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The Protective Effects of Cyclodextrins against the Oxidation of Methyl Orange by Singlet Oxygen

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The degradation of Methyl Orange (MO) by singlet oxygen was inhibited by the addition of α-, β-, and γ-cyclodextrins (CD's) to an aqueous solution. The protective effect of the CD's against the MO oxidation decreased in this order; α->γ-$\gtrsim$β-CD. From the relation between the rate constant of MO oxidation and the free MO concentration, evaluated by using the formation constants of CD's with MO, it was found that the MO included by α- and γ-CD's is not decomposed by singlet oxygen, whereas the MO included by β-CD is oxidized by singlet oxygen. The inner cavity of α- or γ-CD well fit for the inclusion of one or two MO molecules respectively, and the access of singlet oxygen to the azo group of the included MO molecule is sterically inhibited. The finding that β-CD has smaller enthalpy and larger entropy changes relevant to the formation of the MO–CD complex than those of α- and γ-CD's suggested the less tight inclusion of one MO molecule in the cavity of β-CD. Such a loose inclusion of MO by β-CD resulted in the transient exposition of the azo group of the MO molecule to the bulk phase: the attack of the singlet oxygen on the azo group followed.

Cyclodextrins (CD's) are oligosaccharides which are capable of forming inclusion complexes with a variety of guests by accommodating them in their relatively nonpolar cavities.[1,2] The most promising fields for the application of their inclusion complexes are the pharmaceutical industry, the food industry, and the production of organic chemicals in general.[2] One of the most important potential applications of inclusion complexes is the protection of guests against oxidation.[3–7] The stability of incorporated unsaturated fatty acids,[4,5] vitamins,[3,4,7] and so on has been studied by numerous workers. Irie et al., for example, reported the protective effects of the CD's against the oxidation of guests by active oxygen, singlet oxygen, but the protective mechanism was not made clear.[8]

In our previous studies, the photooxidations of Methyl Orange (MO) sensitized by water-insoluble tetraphenylporphyrin and water-soluble tetraphenylporphyrintrisulfonic acid salt (TPPS) have been studied under aerobic conditions in a variety of micellar[9,10] and liposomal solutions[11] and mixed organic solvents.[9] It was found that the oxidation of MO is mediated by singlet oxygen;[12] the photooxidation proceeds predominantly through Type II.[13] MO is capable of forming complexes with CD's,[14,16] and MO is favorable for studies of the effects of CD's on the oxidation by singlet oxygen, In the present study, we investigated the oxidation of MO photosensitized by TPPS in the presence of CD's, also, the protective effects of CD's on the oxidation are discussed in terms of the inaccessibility of singlet oxygen to the MO molecules which are included by the CD's.

Experimental

The *meso*-α,β,γ,δ-tetraphenylporphyrintrisulfonic acid salt tetrahydrate (TPPS) was purchased from Dojindo Laboratories. The absorption bands and their molar extinction coefficients in a 10 mM (1 M=1 mol·dm^{-3} in this paper) buffer solution (pH 7.5) agreed with the reported values.[16] The TPPS in methanol was stored below 4 °C as a stock solution. The MO was obtained from Wako Pure Chemical Industries, Ltd. The 2-amino-2-hydroxymethylpropane-1,3-diol (Tris) was used as received. Doubly distilled water was used to make all solutions.

The samlpe solution was prepared as follows. The methanol of the TPPS stock solution was evaporated in vacuo, after which the residue was dissolved in a 1 mM Tris–HCl buffer solution containing 21 μM MO. The pH of the aqueous solution was maintained at 7.3. Finally, a 1 μM TPPS solution was obtained.

Irradiation experiments were carried out by using a Kondo-Sylvania 1 kW tungsten-halogen lamp. A Toshiba KL-41 interference filter (transmittance characteristics; λ_{max}= 412.0 nm and half wavelength=15.0 nm) was used to obtain monochromic light. The intensity of the light incident upon the reaction cell was 3.22×10^{15} quanta·s^{-1}, as determined by ferrioxalate chemical dosimetry.[17] The sample solution contained in a quartz cell was kept at 25 °C by circulation of the thermostated water and was stirred well with a magnetic stirrer during irradiations.

The absorption spectra of TPPS and MO in the aqueous solution overlap each other in the region of 360—500 nm, as is shown in Fig. 1. The decomposition of MO was monitored with the decrease in the absorbance at 496 nm, where the absorbance of TPPS is trivial. Since the ratio of the extinction coefficient of MO at 412 nm to that at 496 nm was is constant, the time course of the absorbance of MO at 412 nm was obtained from that at 496 nm. The absorption spectra were measured by using a Shimadzu UV-265FW spectrophotometer at 25 °C.

Results and Discussion

MO and TPPS were both decomposed with the increase in the irradiation time, because both TPPS and MO have strong absorptions at the excitation wavelength (412 nm), as is shown in Fig. 1. The decomposition of both dyes led to a change in the amounts of photons absorbed by TPPS in a unit of

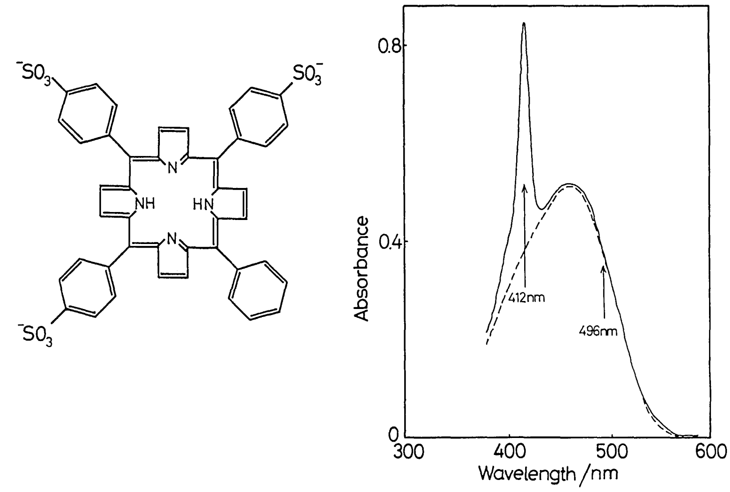

Fig. 1. Chemical structure of TPPS and absorption spectra of 21 μM MO in 1 mM Tris-HCl buffer solution (pH 7.3) with 1.0 μM TPPS (—) and without TPPS (----).

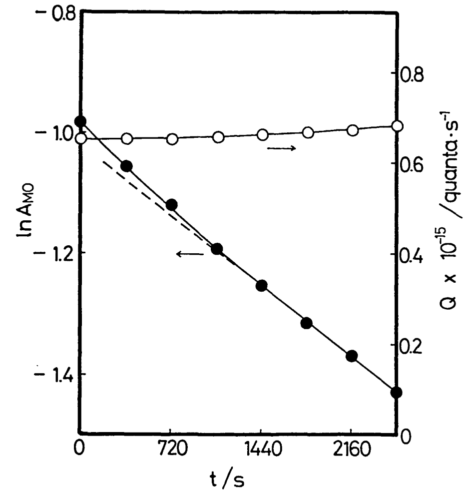

Fig. 2. Time course of logarithm of absorbance of MO at 412 nm (ln A_{MO}, ●) and amounts of photons absorbed by TPPS in a unit time (Q, ○).

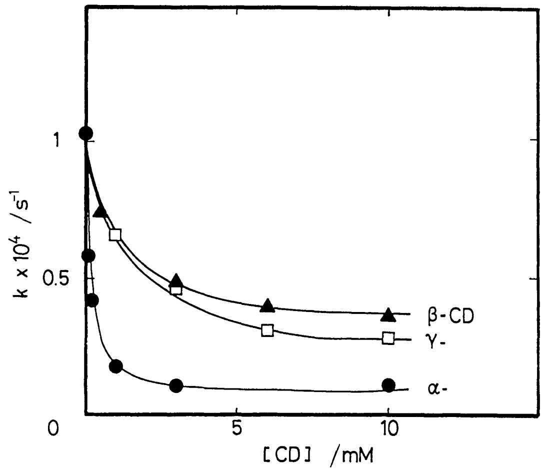

Fig. 3. The apparent rate contant k as a function of the CD concentration. Symboles, ●: α-CD, ▲: β-CD, and □: γ-CD.

time (Q) during irradiation. Figure 2 represents the plots of Q as a function of the irradiation time (t). Here, the Q values were evaluated by means of Eq. 1:[10]

$$Q = I_0 \frac{A_{\mathrm{TPPS}}}{A_{\mathrm{T}} \ln 10}\left(1 - 10^{-LA_{\mathrm{T}}}\right) \quad (1)$$

where $A_{\mathrm{T}} = A_{\mathrm{TPPS}} + A_{\mathrm{MO}}$. Here, A_{T}, A_{TPPS}, and A_{MO} are the total absorbance and the absorbance of TPPS and MO at 412 nm respectively. The I_0 quantity is the intensity of light incident upon the cell, and L is the light-path length of the cell. Fortunately, however, as is shown in Fig. 2, the Q values scarcely changed during irradiation, because a decrease in A_{TPPS} causes a decrease in Q, but a decrease in A_{MO} results in an increase in Q. As the change in the Q values was less than 5% of the initial value after a 42-min irradiation, the change in the Q value was not taken into account in the present study.

As is shown in Fig. 2, there was a linear relationship between ln A_{MO} and the irradiation time t except for an initial period of the irradiation time. The slope of the straight line gave the apparent rate constant k. The degradation rate of MO is given by:

$$\frac{\mathrm{d[MO]_T}}{\mathrm{d}t} = k\mathrm{[MO]_T} \quad (2)$$

and

$$\ln\mathrm{[MO]_T} = \ln\mathrm{[MO]_0} - kt, \quad (3)$$

where $\mathrm{[MO]_T}$ and $\mathrm{[MO]_0}$ are the total and initial concentrations of MO respectively.

Figure 3 shows the k values as a function of the CD concentrations. The k values decrease with an increase in the CD concentration, indicating that the additions of the CD's result in protection against the MO oxidation. The protective effects are, however, different in the three CD's decreasing in this order; α->γ-$\gtrsim$$\beta$-CD. It was been known that MO forms inclusion complexes with various CD's[14,15] Therefore, it may be considered that the formation of the inclusion complex contributes to the protective effects against the MO oxidation.

The formations of inclusion complexes between CD's and MO has been investigated by many workers. Matsui et al. found the dissociation constant of the MO-α-CD complex in 0.1 M H_2SO_4 (pH=1.2, ion strength, μ=0.5) at 25 °C, but their experimental conditions were quite different from ours.[15] Hirai et al. reported the dissociation constants of MO complexes with β- and γ-CD's in a phosphate buffer (pH=7.0, ionic strength, μ=0.1) at 20 °C.[18] However, no data on the dissociation constant of MO-α-CD complex have been reported. Therefore, the formation constants were measured under the present conditions. Hirai and his coworkers suggested that the absorption peak of MO shifts from 463 nm to a shorter wavelength with an increase in the CD concentration and ascribed the hypsochromic shifts to the formation of inclusion complexes of MO with the CD.[18] The formation constants were determined by measuring these absorption changes. (TPPS does not interact with the CD's because the absorption and fluorescence spectra and the fluorescence yield of TPPS scarcely change between the solutions with and without the CD's.)

Hirai and his coworkers also suggested α- and β-CD's form 1:1 (CD:MO in molar ratio) complexes, while γ-CD forms a 1:2 complex.[18] If the concentration of MO is significantly smaller than that of CD, the two formation constants K_{f1} and K_{f2} can be represented by Eqs. 4 and 5.[18]

In the case of a 1:1 complex (α- and β-CD's),

$$K_{f1}=\frac{[\mathrm{CD{-}MO}]}{[\mathrm{CD}][\mathrm{MO}]} \tag{4-a}$$

and

$$\frac{1}{\Delta A}=\frac{1}{[\mathrm{MO}]\Delta\varepsilon K_{f1}}\cdot\frac{1}{[\mathrm{CD}]}+\frac{1}{[\mathrm{MO}]\Delta\varepsilon} \tag{4-b}$$

In the case of a 1:2 complex (γ-CD),

$$K_{f2}=\frac{[\mathrm{CD{-}(MO)_2}]}{[\mathrm{CD}][\mathrm{MO}]^2} \tag{5-a}$$

and

$$\frac{1}{\Delta A}=\frac{1}{[\mathrm{MO}]^2\Delta\varepsilon K_{f2}}\cdot\frac{1}{[\mathrm{CD}]}+\frac{2}{[\mathrm{MO}]\Delta\varepsilon} \tag{5-b}$$

Here, ΔA and $\Delta\varepsilon$ are, respectively, the differences in the absorbance and in the molar extinction of MO at 495 nm, between the solutions without and those with CD's. The concentration of the latter being significantly higher than that of MO. In the case of γ-CD, the formation of a 1:1 complex was ignored, because K_{f1} is much smaller than K_{f2}.[18] The ΔA values at various concentrations of CD were measured at 21 µM MO. Figure 4 shows the $1/\Delta A$ values as a function of the [CD] according to Eqs. 4-b and 5-b: linear relations were obtained. The formation constants evaluated from the slopes and the intercepts to the ordinate are given in Table 1. From the formation constants thus obtained, the concentration ratio of the free MO to the total MO were calculated at 10 mM CD: they are presented in Table 2. The concentration of the included MO decreases in this order: α->β->γ-CD. This order is different from that of the protective effect

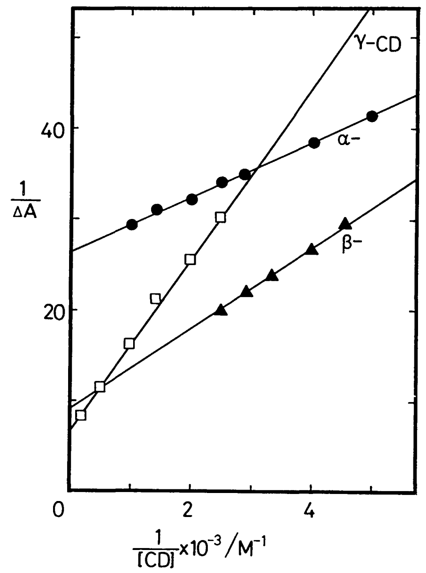

Fig. 4. The $1/\Delta A$ values as a function of the CD concentration according to Eqs. 4-b and 5-b. Symboles: see Fig. 3.

Table 1. Formation Constants of the Inclusion Complexes between MO and CD's at Various Temperatures

Temperature	K_{f1}		K_{f2}
°C	10^3 M^{-1}		10^7 M^{-2}
	α-CD	β-CD	γ-CD
15.0	1.4_7	2.6_3	2.8_2
20.0	1.1_3	2.3_4	2.3_5
25.0	0.85_5	2.1_0	1.8_9
30.0	0.70_4	1.8_9	1.2_2

Table 2. Concentration Ratio of Free MO to Total MO

CD's	α	β	γ
$\frac{[\mathrm{MO}]_{\mathrm{free}}{}^{a)}}{[\mathrm{MO}]_{\mathrm{T}}}$	0.012	0.015	0.31

a) At 10 mM CD, 21 µM MO, and 25 °C.

of the CD's against the MO oxidation, as is shown in Fig. 3. On the assumption that only free MO is decomposed by singlet oxygen, the decomposition rate of MO is given by:

$$-\frac{d[\mathrm{MO}]_\mathrm{T}}{dt} = k'[{}^1\mathrm{O_2}][\mathrm{MO}]_\mathrm{free}, \tag{6}$$

because the production processes of singlet oxygen are much faster than the oxidation process of MO by singlet oxygen[9–13] and the rate of reaching equilibrium between free and included MO is much faster than the degradation rate of MO. Here, k' is the second-order rate constant, while $[\mathrm{MO}]_\mathrm{free}$ is the free MO concentration.

Equation 6 may be rewritten as:

$$-\frac{d[\mathrm{MO}]_\mathrm{T}}{dt} = \frac{k'[{}^1\mathrm{O_2}][\mathrm{MO}]_\mathrm{free}}{[\mathrm{MO}]_\mathrm{T}}[\mathrm{MO}]_\mathrm{T} \tag{7}$$

As can be seen by comparing Eqs. 2 and 7, the apparent rate constant k corresponds to k' $[{}^1\mathrm{O_2}][\mathrm{MO}]_\mathrm{T}$:

$$k = k'[{}^1\mathrm{O_2}]\frac{[\mathrm{MO}]_\mathrm{free}}{[\mathrm{MO}]_\mathrm{T}} = k''\frac{[\mathrm{MO}]_\mathrm{free}}{[\mathrm{MO}]_\mathrm{T}} \tag{8}$$

where $k''=k'$ $[{}^1\mathrm{O_2}]$ and where k'' is a constant, for the complex formation of CD's with oxygen is negligibly small and $[{}^1\mathrm{O_2}]$ is independent of the CD concentration.[19] As can be seen from Eq. 8, if only free MO is decomposed by singlet oxygen, the k values may be expected to be proportional to $[\mathrm{MO}]_\mathrm{free}/[\mathrm{MO}]_\mathrm{T}$. Figure 5 presents the plots of the k values against the $[\mathrm{MO}]_\mathrm{free}/[\mathrm{MO}]_\mathrm{T}$ values calculated from the K_f1 or K_f2 values in Table 1. Taking the small experimental errors at the large values of $[\mathrm{MO}]_\mathrm{free}/[\mathrm{MO}]_\mathrm{T}$ into consideration (experimental errors: within ±5%), the correlation line was drawn, assuming the points in the low CD concentrationto be accurate. In the α- and γ-CD's, the straight line thus obtained passes through both the point of origin and the experimental point without the CD's. This fact indicates that the MO included by α- and γ-CD's is not decomposed by singlet oxygen and that only free MO is oxidized. On the other hand, in the case of β-CD, the plots deviate upward from the experimental line of the α- and γ-CD's. The line does not pass through a point of origin and has a positive intercept on the ordinate, where all MO molecules should be included by the CD's. This leads to the important consequence that free MO and also the MO included by β-CD are appreciably oxidized by singlet oxygen.

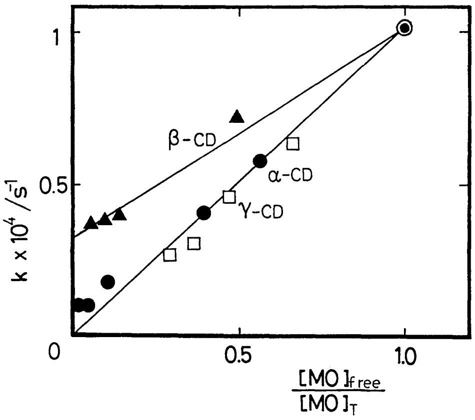

Fig. 5. The k values as a function of $[\mathrm{MO}]_\mathrm{free}/[\mathrm{MO}]_\mathrm{T}$. The $[\mathrm{MO}]_\mathrm{free}/[\mathrm{MO}]_\mathrm{T}$ values at 21 μM MO were evaluated from the formation constants K_f1 and K_f2 in Table 1. Symboles: see Fig. 3.

Griffiths et al. investigated the oxidation mechanism of tautomeric azo dyes by singlet oxygen; they suggested that the degradation of the azo dyes to be ascribed to the attack of singlet oxygen as the azo group in the dye molecules.[20] The oxidation of MO by singlet oxygen may also be considered to proceed in accordance with their porposed mechanism.

The inner cavity of the α- or γ-CD's is suitable for the inclusion of one or two MO molecules respectively. The fitness of the MO in the cavities of α- and γ-CD's is good, and the access of singlet oxygen to the azo group of the MO molecule is sterically protected. On the other hand, β-CD has a sufficiently large inner cavity to include a MO, molecule, while the cavity of β-CD is too small to include two MO molecules. We can write the complex formation reaction of β-CD with MO by the following two step reaction,[21] in the cases of the α- and β-CD's/MO system:

$$\mathrm{MO} + \mathrm{CD} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\mathrm{MO}\cdots\mathrm{CD}] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{MO\text{–}CD} \tag{9}$$

where $[\mathrm{MO}\cdots\mathrm{CD}]$ and MO–CD are intermediate and stable complexes respectively. Here, K_f1 is related to k_1, k_{-1}, k_2, and k_{-2} as follows:[21] $K_\mathrm{f1}=(k_1/k_{-1})+(k_1/k_{-1})\times(k_2/k_{-2})$. In contrast to the α-CD complex, the k_2 value of the MO–β-CD complex may be comparable the k_{-1} values, because the size of the cavity of β-CD is larger than that of α-CD. Therefore, the lifetime of the intermediate complex becomes longer. In other words, the MO molecule in the MO–β-CD complex has a hindered translational freedom in addition to the rotational freedom.[21] It may be expected that the inclusion of a MO molecule in the cavity of β-CD will be loose. The thermodynamic parameters in unitary

Table 3. Thermodynamic Parameters Relevant to the Inclusion of MO by CD's in an Aqueous Solution in a Unitary Change

CD's	ΔG_0[a)]	ΔH_0	ΔS_0
	kcal mol⁻¹	kcal mol⁻¹	eu
α	−5.4	−8.6	−11
β	−4.5	−3.8	+2.5
γ	−9.9	−12	−8.1

a) At 25 °C.

changes based on the inclusion of a MO by CD's are presented in Table 3. The changes in the free-energy (ΔG_0), enthalpy (ΔH_0), and entropy (ΔS_0) were calculated from the data in Table 1 by using:

$$\Delta G_0 = -RT\ln K_{f1} \quad (\text{or } K_{f2}), \tag{10-a}$$

$$\Delta H_0 = \Delta G_0 + T\Delta S_0 \tag{10-b}$$

and:

$$\Delta S_0 = -(\partial \Delta G_0 / \partial T)_p. \tag{10-c}$$

The formation constants, K_{f1} and K_{f2}, in Eq. 10-a are expressed in the molarity scale and are shown in Table 1. R is the gas constant, and T, the absolute temperature. It may be seen that, in the inclusions of MO by α- and γ-CD's, the enthalpy change acts as a dominant driving force and the ΔS_0 values are negative. On the other hand, in the inclusion of MO by β-CD, the enthalpy and entropy changes contribute equally to the complex formation and the ΔS_0 value is positive. The positive value of ΔS_0 for the β-CD complex formation seems to be involved in the appreciable liberation of high-energy water in the cavity from the larger cavity of β-CD, as compared with α-CD, because both form the 1:1 complex. These thermodynamic quantities reflect the presence of an unstable intermediate complex in the MO/β-CD system.

Szejtli suggested that MO molecule is less tightly included in the cavity of β-CD.[23] By their NMR measurements Suzuki and her colleagues found the fast rotational motion of a MO molecule included in the cavity of β-CD.[24] These facts agree well with our results. Thus, the loose inclusion of a MO molecule in the cavity of β-CD gives rise to the transient exposition (intermediate complex) of the azo group of the MO molecule to the bulk phase, as shown in Fig. 6a. This leads to the decomposition by the attack of singlet oxygen on the azo gruop.

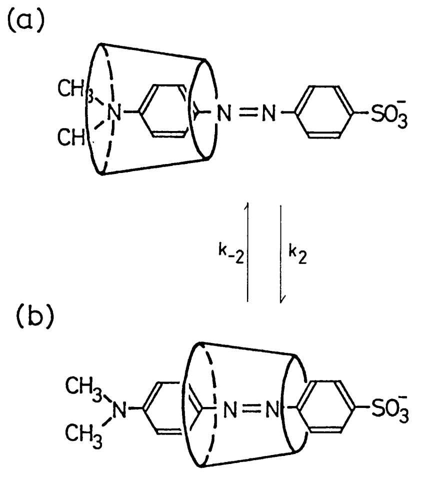

Fig. 6. The loose inclusion of MO in the cavity of β-CD.

In the present study, it was found that the CD's sterically protect MO against the attack of active singlet oxygen by including a MO molecule in their cavities. The CD's have been utilized to protect many substances from oxidation. In this situation, not only the formation constants but also the fitness between the inner cavities of CD's and the size of the guest substrates must be taken into consideration.

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