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COMPLEX FORMATION OF WATER-SOLUBLE PORPHYRIN WITH CYCLODEXTRIN

Hidefumi HIRAI,* Naoki TOSHIMA, Shinji HAYASHI, and Yasufumi FUJII
Department of Industrial Chemistry, Faculty of Engineering,
The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

Interactions of monomer forms of protoporphyrin IX, deuteroporphyrin IX, hematoporphyrin IX, and coproporphyrin III, respectively, with α- and γ-cyclodextrin in 0.002 mol·dm^{-3} NaOH aqueous solution are indicated by change of electronic spectrum and induction of circular dichroism. Dimerization constant of porphyrin and complex formation constant of porphyrin with cyclodextrin are also determined from the electronic spectra.

Gamma-cyclodextrin can include a large molecule (anthracene,[1] congo red,[2,3] and bis-(4-biphenylmethyl)-ammonium chloride[4]) or two rather small molecules (sodium α-naphthylacetate,[5] pyrene,[6,7] crystal violet,[3] methylene blue,[3] methyl orange,[3] and chlorpromazine[8]) in its large cavity of hydrophobic domain.[9] In the oxygen carrier in blood, hemoglobin, the porphyrin-iron(II) complex is stabilized by inclusion with the hydrophobic domain of globin protein.[10] From these points of view, we have at first investigated the inclusion complex formation of water-soluble porphyrins with γ-cyclodextrin.

Porphyrins are generally known to aggregate each other in an aqueous solution. Figure 1 shows the concentration dependence of the electronic spectrum of hematoporphyrin IX in a 0.002 mol·dm^{-3} aqueous solution of sodium hydroxide at 20°C. Dilution of the solution of hematoporphyrin IX affords the decrease of the band at 370 nm which is assigned to the dimer of the porphyrin, and the increase of the peak at 393 nm which is assigned to the porphyrin monomer when the spectra are taken under the conditions that the ratio of the intensity scale to the concentration is kept constant. The analysis of this spectral change with concentration according to Karns et al.[11] has given the formation constant of the hematoporphyrin dimer to be 4.9×10^4 dm^3·mol^{-1}, which means that the monomer and the dimer coexist in equilibrium at the ratio of 36 : 64, in the 4×10^{-5} mol·dm^{-3} aqueous solution of hematoporphyrin IX.

Interaction of hematoporphyrin with cyclodextrin has been examined by adding cyclodextrin to the above solution. The change of the electronic spectrum and circular dichroism of hematoporphyrin IX on addition of γ-cyclodextrin is shown in Fig. 2, where increase of the monomer peak at 396 nm is observed. The wave length of maximal absorption assigned to the monomer is shifted from 393 nm in Fig. 1 to 396 nm in Fig. 2 by addition of γ-cyclodextrin. These results suggest the complex formation of the hematoporphyrin monomer with γ-cyclodextrin. This complex formation has been further supported by the induction of circular dichroism of a

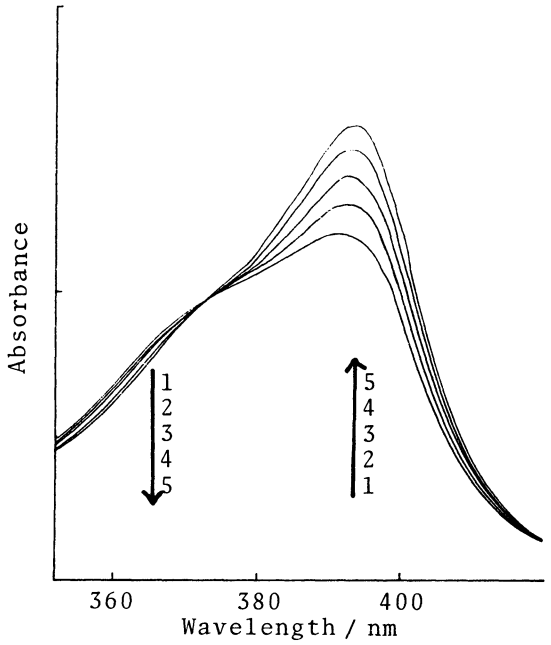

Fig. 1 Electronic spectra of hematoporphyrin IX in 0.002 mol·dm^{-3} NaOH aq. at the concentrations of 2.0×10^{-5} (1), 1.0×10^{-5} (2), 5.0×10^{-6} (3), 2.5×10^{-6} (4), 1.25×10^{-6} mol·dm^{-3} (5). Intensity Scale / Concentration = Constant.

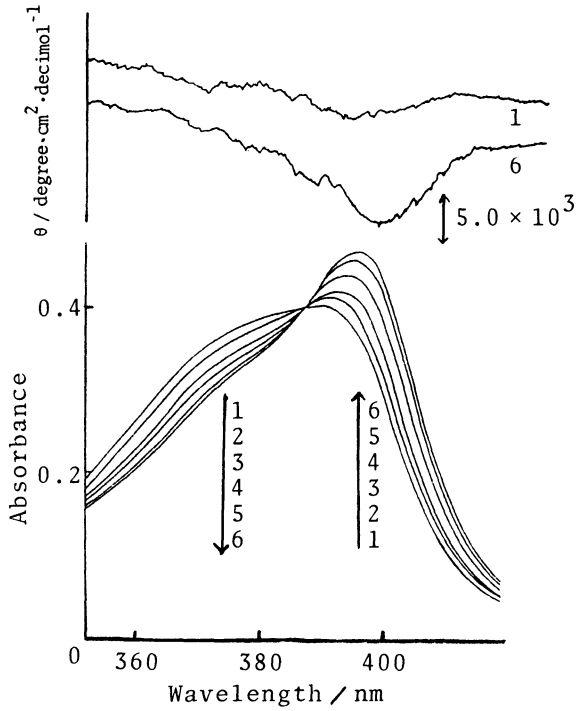

Fig. 2 Electronic and circular dichroism spectra of hematoporphyrin IX (4×10^{-5} mol·dm^{-3}) in the absence (1) and presence of γ-cyclodextrin at the concentration of 0.5 (2), 1.0 (3), 2.0 (4), 3.0 (5), and 4.0×10^{-2} mol·dm^{-3} (6) in 0.002 mol·dm^{-3} NaOH aq.

TABLE 1. WAVE LENGTH OF MAXIMAL ABSORPTION, λ_{max}, IN ELECTRONIC SPECTRUM AND MAXIMUM APPARENT MOLAR ELLIPTICITY, θ, IN CIRCULAR DICHROISM OF PORPHYRIN IN THE ABSENCE AND PRESENCE OF CYCLODEXTRIN (CyD)[a)]

Porphyrin	2,4-Disubstituent	λ_{max}/nm			$\theta \times 10^{-3}$ degree·cm^2·decimol^{-1}	
		Non	α-CyD[b)]	γ-CyD[c)]	α-CyD[d)]	γ-CyD[c)]
Proroporphyrin IX	Vinyl	378	383 396sh	385 396sh	—[e)]	—[e)]
Deuteroporphyrin IX	Hydrogen	372 392sh	392	390	7.5	8.4
Hematoporphyrin IX	α-Hydroxyl	376sh 393	395	396	5.9	9.4
Coproporphyrin III	Propanoic acid	393	396	397	11.9	16.9

a) [porphyrin] = 4×10^{-5} mol dm^{-3} in 0.002 mol·dm^{-3} NaOH aq. Shoulder peaks are singed by sh. b) [α-CyD] = 5.35×10^{-2} mol·dm^{-3}. c) [γ-CyD] = 4.0×10^{-2} mol·dm^{-3}. d) [α-CyD] = 4.0×10^{-2} mol·dm^{-3}. e) not determined.

hematoporphyrin monomer with addition of γ-cyclodextrin at 380 - 410 nm as shown in the upper part of Fig. 2.

The similar change of electronic spectrum and induction of circular dichroism have been observed by addition of α-cyclodextrin instead of γ-cyclodextrin. However, these changes could not be observed by addition of glucose which is a monomeric unit of cyclodextrin. This fact indicates that the ring structure of cyclodextrin plays an important role for the complex formation of porphyrin with cyclodextrin. The similar changes in electronic spectra and in circular dichroism have been observed for protoporphyrin IX, deuteroporphyrin IX, and coproporphyrin III. The results are summarized in TABLE 1.

The equilibrium constant, K_c, for the complex formation of porphyrin with cyclodextrin can be calculated by the following way. A porphyrin monomer is equilibrated with its dimer as shown in Eq. 1, as well as with cyclodextrin to form the complex as shown in Eq. 2. From these equilibria, Eqs. 3 and 4 can be derived where K_d is the equilibrium constant for dimer formation, and P, P_2, C, and P-C indicate a porphyrin monomer, a porphyrin dimer, cyclodextrin, and the complex of porphyrin with cyclodextrin, respectively.

$$2\,P \underset{}{\overset{K_d}{\rightleftharpoons}} P_2 \qquad (1)$$

$$P + C \overset{K_c}{\rightleftharpoons} P\text{-}C \qquad (2)$$

$$K_d = \frac{[P_2]}{[P]^2} \qquad (3)$$

$$K_c = \frac{[P\text{-}C]}{[P][C]} \qquad (4)$$

Since porphyrin exists as monomer, dimer, and the complex with cyclodextrin under the equilibrium condition, the initial concentration of porphyrin, $[P_o]$, can be represented by Eq. 5, and the absorbance of the equilibrated solution can be indicated by Eq. 6 by using molar absorption coefficient, ε, of each species.

$$[P_o] = [P] + 2[P_2] + [P\text{-}C] \qquad (5)$$

$$\frac{d}{1} = \varepsilon_p[P] + \varepsilon_{p_2}[P_2] + \varepsilon_{p\text{-}c}[P\text{-}C] \qquad (6)$$

From Eqs. 3, 4, 5, and 6, $(K_c[C] + 1)$ has been obtained as the function of d, K_d, ε_p, ε_{p_2}, and $\varepsilon_{p\text{-}c}$ as shown in Eq. 7.

$$K_c[C] + 1 = f(d, K_d, \varepsilon_p, \varepsilon_{p_2}, \varepsilon_{p\text{-}c}) \qquad (7)$$

Here, the equilibrium constant for dimer formation, K_d, and molar absorption coefficient of monomer and dimer, ε_p and ε_{p_2}, respectively, can be obtained from the dilution experiments of porphyrin like Fig. 1. Absorbance, d, and the concentration of cyclodextrin, [C], can be obtained from the experimental conditions.

TABLE 2. DIMERIZATION CONSTANT, K_d, OF PORPHYRIN AND COMPLEX FORMATION CONSTANT, K_c, OF PORPHYRIN WITH CYCLODEXTRIN (CyD)[a)]

Porphyrin	K_d / $dm^3 \cdot mol^{-1}$	K_c / $dm^3 \cdot mol^{-1}$	
		α-CyD	γ-CyD
Deuteroporphyrin IX	8.7×10^4	16	—[b)]
Hematoporphyrin IX	4.9×10^4	35	73
Coproporphyrin III	0.1×10^4	23	64

a) in a 0.002 $mol \cdot dm^{-3}$ NaOH aqueous solution at 20°C.
b) not determined.

The best fitness of the data to the Eq. 7 gives the equilibrium constant for the complex formation, K_c. The data of K_d and K_c are summarized in TABLE 2.

The results in TABLE 2 indicate that 1) the interaction of porphyrin with cyclodextrin increases in the order: deuteroporphyrin < coproporphyrin < hematoporphyrin, and 2) γ-cyclodextrin interacts with each porphyrin more strongly than α-cyclodextrin.

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