SYNTHESIS OF CYCLODEXTRIN APPENDED PORPHYRIN AND ITS GUEST-INDUCED SUPER SLOW MULTICONFORMATIONAL CHANGES

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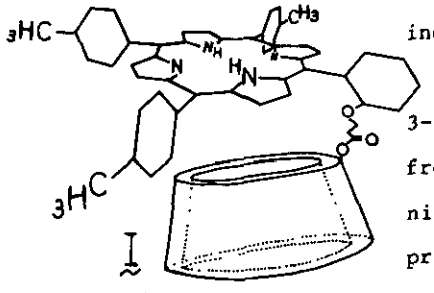
Abstract —— Porphyrin and cyclodextrin were flexibly linked through one covalent bond and some of its properties in solution, especially, guest-induced, super slow diverse CD changes in aqueous solution are reported.

Up to today, a variety of heme model compounds have been prepared. For example, so-called "picket fence",[1] "capped",[2] and "cyclophane"[3] porphyrins have been reported in an attempt to make oxygen carrier models. In particular, the iron complex of picket fence porphyrin was an eminent model; it oxygenated reversibly at lower pressure of oxygen than hemoglobin does. However, the conclusive limit of these compounds as hemoglobin models was that they were insoluble in water which is the solvent of natural systems. Accordingly, we designed to synthesize a water-soluble oxygen carrier model. Thus as the first step, cyclodextrin appended porphyrin, I (double bonds are deleted for clarity), and its iron complex, II, were prepared. Unfortunately, however, II did not reveal the expected function. But here, we wish to describe the synthesis of I and some of its properties in solution. Because it showed an intriguing behavior on cyclohexanol addition which may correlate to substrate-induced conformational changes of enzymes.



I

I was prepared according to the following route. 3-(2-Formyl)phenoxypropionic acid (III) was synthesized from salicylaldehyde and β-chloropropionic acid under nitrogen in 42% yield by the procedure to make 3-phenoxypropionic acid[4,5]. *meso*-α-*o*-(2-Carboxy-1-ethoxy)phenyl-β,γ,δ-tri-*p*-tolylporphyrin (IV) was obtained from the mixed condensation of *p*-tolu-

aldehyde, III and pyrrole in refluxing propionic acid based on the Adler-Longo porphyrin condensation procedure[6] and subsequent fractionation by silica-gel chromatography (2.5%). To prepare I, potassium salt of IV was reacted with monotosylated (primary alcohol site) β-cyclodextrin[7] in the presence of 1 to 2 equivalent(s) of 18-crown-6 in dimethylformamide at 90 °C. After 24 h, the solvent was removed by evaporation, and the residue was washed with acetone, filtered and fractionated on a Sephadex G-25 column twice using H_2O as eluent (6%). The ^1H NMR (DMSO-d^6) data were in complete accord with the suggested structure; δ-NH at -2.84(2H,s), aromatic at 7.39-8.12(16H,m), β-pyrrole at 8.80 (8H,s), C_2,C_3-OH at 5.62(14H,s), C_1-H at 4.80(7H,s), C_6-OH at 4.41(6H,s), and others at 4.2-1.9(55H,m). IR: 3350, 2925, 1730(ester), 1155, 1080, 1027, 844, 752, 700 cm^{-1}. TLC(silica-gel, Merck Art.5723): Rf=0.62(n-BuOH:EtOH:H_2O=5:4:3 v/v/v). Various kinds of of spectra on I in DMSO or in water are shown in Fig.1. Absorption and MCD spectra indicate that I is monomeric[8] in DMSO, while spectral broadening in the Soret and red shifting of visible bands suggest I being aggregative in water[9].

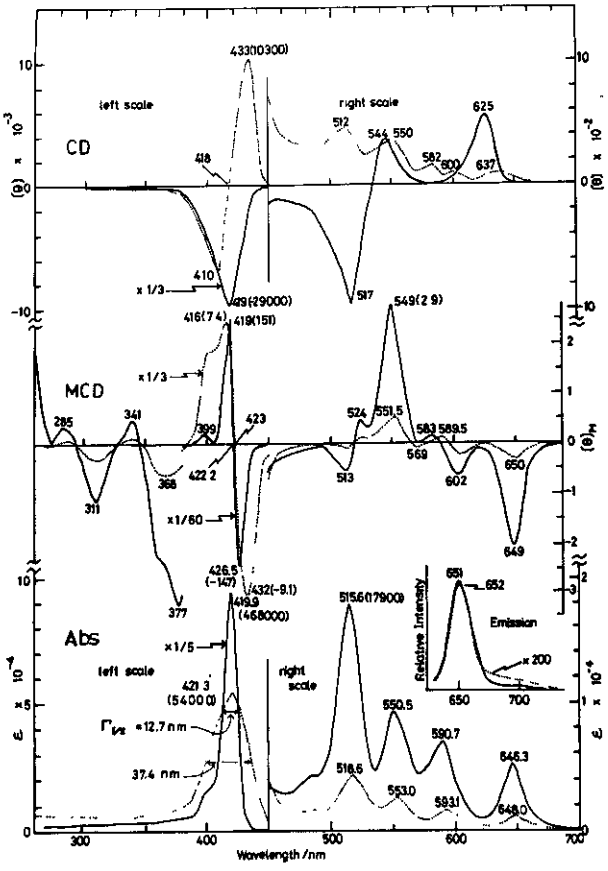


Fig.1 Absorption(bottom), MCD(middle), CD(top), and emssion(inset) spectra of I in DMSO(——) and in 0.05 M TRIS buffer(pH 7.3)(------) at 23±1 °C. Concentrations; 3.72 x 10^{-5} M in DMSO except that in emission spectrum(1.46 x10^{-4} M), and 1.46 x 10^{-4} M in water. Taking into account of the aggregation problem cells were changed from 1 to 100 mm length keeping concentrations constant. In emission spectra, excitation was at 467 nm. Magnetic field;11700 gauss. In order to maximize signal to noise ratio, all spectra are the average of at least triplicate runs, the cells being reversed in the second run. Unit; $[\theta]_M$=deg $cm^2dmol^{-1}{}_G{}^{-1}$. $[\theta]$=degcm^2dmol^{-1}.

A dramatic CD change of I in water observed by the addition of optically inactive guest, cyclohexanol, is shown in Fig.2. While cyclohexanol concentrations were small, CD changed slightly. But when [cyclohexanol]=0.224 M was attained, an abrupt and complex CD change began to appear. It changed continuously and extremely slowly (in the order of A to D) and

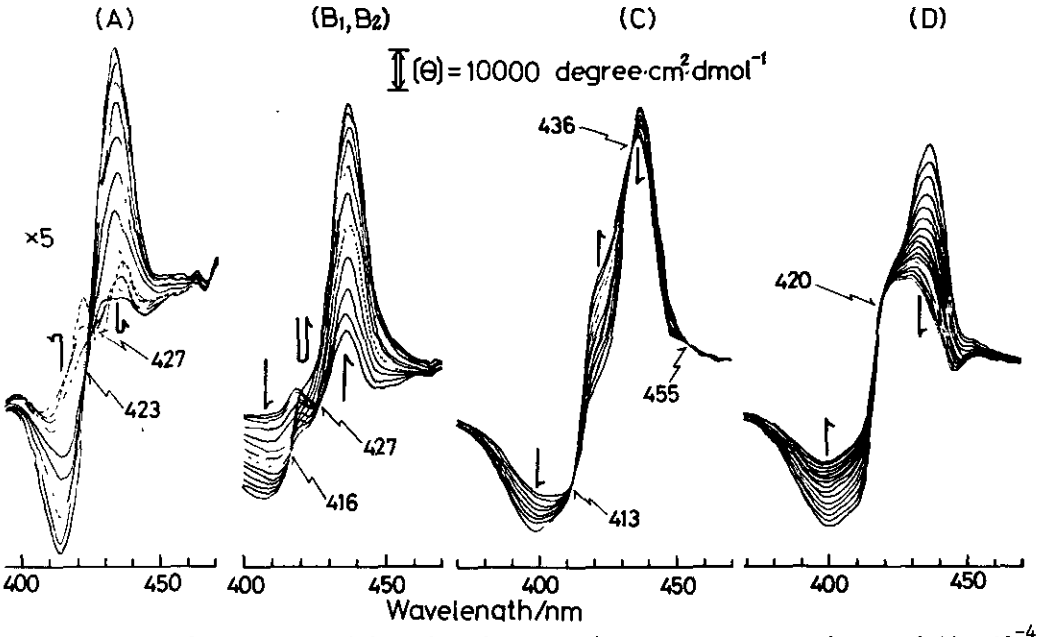


Fig.2 CD changes of $\underset{\sim}{I}$ by the addition of cyclohexanol in water. Concentrations: $\underset{\sim}{I}$=1.46 x 10^{-4} M, (however, at the end of experiments, a faint amount of precipitate was recognized). Cyclohexanol =0.224 M. Cell length=1 mm. Time interval: A,B=16.5 min., C,D=28.5 min. Some lines are omitted for clarity. Base lines are neither shown nor corrected. Numbers indicate the wavelengths at the position of isoelliptic points. Bold arrows indicate the direction of CD change. (Vigorous stirring prior to measurement yielded different spectra!).

took almost a full whole day to reach the final state (see also Fig. 3). As judged from this figure, including the initial and the final states in all 6 states appeared accompanying 7 distinct isoelliptic points in the course of consecutive CD change. This indicates that the stepwise CD change proceeded via several of independent states. During this variation, the Soret absorption spectrum also changed manifoldly but their shapes were all far from that of a typical monomeric one. Thus the above mentioned CD change seems to occur retaining aggregative forms, plausively with porphyrin moieties of two $\underset{\sim}{I}$s in sandwich fashion. It is also interesting to note that the critical concentration exists for guest molecule to change $\underset{\sim}{I}$'s conformation. If adamantane carboxylic acid which is too large to be fully accommodated into the cavity of β-cyclodextrin moiety was adopted instead as an additive, such a behavior was not observed. This fact suggests that deep inclusion of guest molecules by β-cyclodextrin moiety was indispensable to and a trigger for the diverse conformational changes.

Figure 3 demonstrates the time dependence of CD intensity at some wavelenghths, replotted from the data in Fig.2. The smooth intensity change during more than 20 h

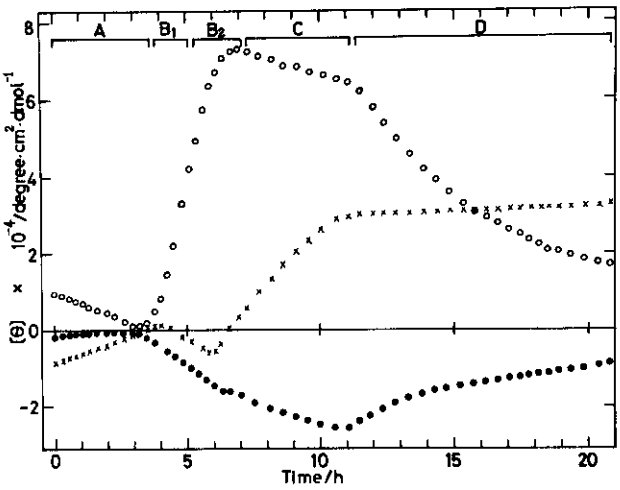


Fig. 3. The time dependence of CD intensity at 400 (●●●), 422 (XXX), and 438 (OOO) nm.

is emphasized. One reason for this is perhaps the steric interaction between a large porphyrin and cyclodextrin structure.

Thus the synthesis of $\underset{\sim}{I}$ and its guest-induced diverse conformational changes are reported. Such a behavior may be to become a model to substrate-induced conformational changes of enzymes.

In order to prepare better oxygen carrier model, the synthesis of "a porphyrin capped cyclodextrin" is now under way in this laboratory.

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REFERENCES

1. J. P. Collman, R. R. Gagne, T. R. Halbert, J. M. Marchon, and C. A. Reed, J. Am. Chem. Soc., 1973, 95, 7868.
2. J. E. Baldwin, T. Klose, and M. Peters, J. C. S. Chem. Commun., 1976, 881.
3. J. E. Linardm, P. E. Ellis, Jr., R. D. Jones, and F. Basolo , J. Am. Chem. Soc., 1979, 101, 4762.
4. A. Kallner, Ber. Deutsch. Chem. Soc., 1924, 57, 204.
5. L. Gresham, J. E. Jansen, F. W. Shaver, R. A. Bankert, W. L. Bears, and G. Marie, J. Am. Chem. Soc., 1949, 71, 661.
6. A. D. Adler, F. R. Longo, L. D. Finarelli, J. Goldmacker, J. Assour, and L. Korsakoff, J. Org. Chem., 1967, 32, 476.
7. L. D. Melton and K. N. Slessor, Carbohydr. Res., 1971, 18, 29.
8. J. C. Sutherland, in ref.1, Vol.3, Chap.4.
9. W. I. White, in ref.1, Vol.5, Chap.7.

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