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EFFECTS OF CYCLODEXTRIN ON ENHANCEMENT FOR CHEMILUMINESCENCE OF THE LUMINOL RELATED COMPOUND

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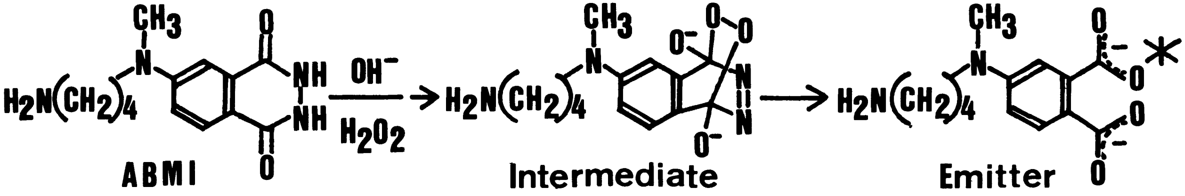
The chemiluminescence of 6-[N-(4-aminobutyl)-N-methyl]amino-2,3-dihydro-1,4-phthalazine dione(aminobutylmethylisoluminol:ABMI) in an alkaline aqueous solution was found to be enhanced by adding β-cyclodextrin(β-CD) to the reaction system. In this case, β-CD exhibited both effects of an increase in the fluorescence quantum yield of the emitter(ϕ_{fl}) and an increase in the fraction of molecules of the intermediate which cross over to the excited state of the product(ϕ_{es}).

Recently, the chemiluminescence(CL) of 5-amino-2,3-dihydro-1,4-phthalazine dione (luminol) or its related compounds, such as aminobutylisoluminol derivatives (ABI derivatives), has been noted as a highly sensitive detection system in the area of analytical chemistry. For example, in the enzymatic method of analysis the CL of luminol has been applied to determine the substrate from the CL intensity emitted by the reaction of luminol and hydrogen peroxide(H_2O_2) enzymatically generated.1) Aminobutylethylisoluminol(ABEI) has also been worthy of note as the CL probe in chemiluminescence immunoassays.2)

To date, ABMI,3) in which the CL intensity is stronger than that of luminol or ABEI, was newly synthesized by the modification of the method of Schroeder et al. for ABEI.4) This was done by the author for the purpose of applying it to analytical chemistry. In the study of the CL characteristics of ABMI, it was found that the CL intensity was profoundly enhanced in the presence of β-CD. This was also observed in the CL of other ABI derivatives. In addition, α-CD exhibited a similar effect in the CL of luminol.

CD is known as a useful catalyst for organic synthesis or as an artificial enzyme model,5,6) but this has yet to be applied to CL. In this paper, the effects of CD on the CL reaction will be estimated from the CL characteristic results, and the course in the CL reaction pathway is significantly related to CD centering around ABMI.

The solvent used for the CL reaction was 0.1 mol·dm^{-3} of Na_2CO_3 solution



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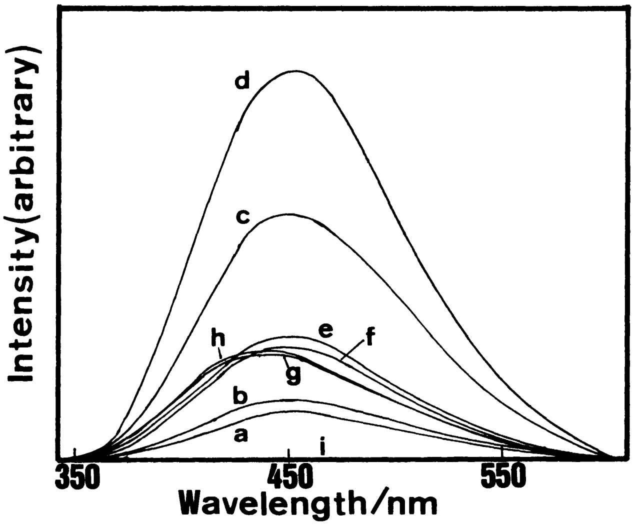

Fig.1. Changes in chemiluminescence spectra (corrected) against additive β-CD concentration, mol·dm^{-3} a=0;b=1.0x10^{-5}; c=2.0x10^{-5};d=5.0x10^{-5};e=7.0x10^{-5};f=1.0x10^{-4}; g=5.0x10^{-4};h=1.0x10^{-3};i=1.0x10^{-3}(ABMI not contained);ABMI=1x10^{-5}mol·dm^{-3}.

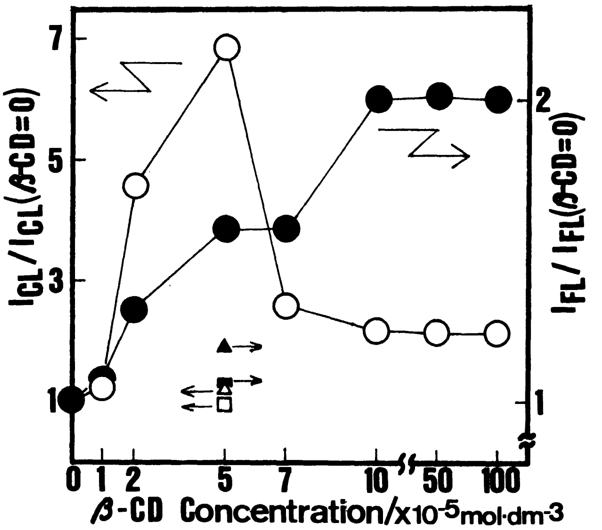

Fig.2. Relationship between the chemiluminescence intensity(I_{CL}), the fluorescence intensity(I_{FL}) and β-CD concentration
△ :α-CD, □:γ-CD.

exclusively, and the concentration of ABMI was constantly kept at 1.0x10^{-5} mol·dm^{-3}. In the present experiments, the CL was initiated by adding a large excess of H_2O_2 without using a catalyst, such as hemin or peroxidase, to simplify the CL reaction system. 125 μdm^3 of 35%H_2O_2 was added to 10 mdm^3 of ABMI solution, and the added H_2O_2 concentration corresponded to 0.13 mol·dm^{-3}. The CL reaction of ABMI seems to follow Eq.1. The CL intensity approached a constant value about 5 min after the addition of H_2O_2, and then it remained almost constant for 10 min. The CL spectra were measured by a Shimadzu spectrofluorophotometer RF-540 while running it with the excitation light off during this period. The wavelength of their excitation spectra maxima was adopted as the excitation wavelength for the measurements of the fluorescence spectra of the total reaction products. The fluorescence(FL) and CL spectra were corrected according to the literature.7) The correction for the intensity of the excitation light was run on the basis of the excitation spectrum of Rohdamine B(3 g·dm^{-3} in ethylene glycol). The ϕ_{fl} was determined relative to the quantum yield of quinine sulfate.7) Every measurement was done at 25 °C.

Figure 1 shows the CL spectra of ABMI at varying β-CD concentration. As can be seen in Fig. 1, the CL intensity (I_{CL}, the area under the CL spectrum) varies depending on the β-CD concentration. The relationship between I_{CL}, the FL intensity (I_{FL}, the area under the FL spectrum) and the β-CD concentration is shown in Fig. 2. I_{FL} gradually increased and approached a plateau value as the β-CD concentration increased. It appears that I_{FL} increases due to the formation of the inclusion compound between β-CD and the emitter (the phthalate dianion).8) However, the behavior of I_{CL} for β-CD concentration does not correspond to that of I_{FL}. I_{CL} was especially enhanced in the region of I_{FL} which did not approach the maximum value. These results suggest that the effects of β-CD on the CL characteristics act on not only I_{FL} of the emitter but also on the intermediate course of the CL reaction pathway. On the other hand, neither α-CD nor γ-CD scarcely influenced on the CL of ABMI.

Figure 3 shows the absorption spectra measured before the CL reaction and the excitation spectra of the total reaction products. By the addition of β-CD, a little red-shift of the peak wavelength and the isosbestic points(310, 350nm) were observed. The isosbestic points indicate a 1:1 equiribrium between ABMI and β-CD. Although there was little difference in the absorption spectra particularly in the relative low additive β-CD concentration to 1.0×10^{-4} mol·dm^{-3}, the excitation spectra were considerably changed being in agreement with the FL results at varying β-CD concentrations. The results shown in Fig. 3 suggest that β-CD forms the inclusion compound with the emitter more easily than ABMI. However, these results do not adequately explain the behaviors of I_{CL} or the effects of β-CD on the intermediate process in the CL reaction pathway.

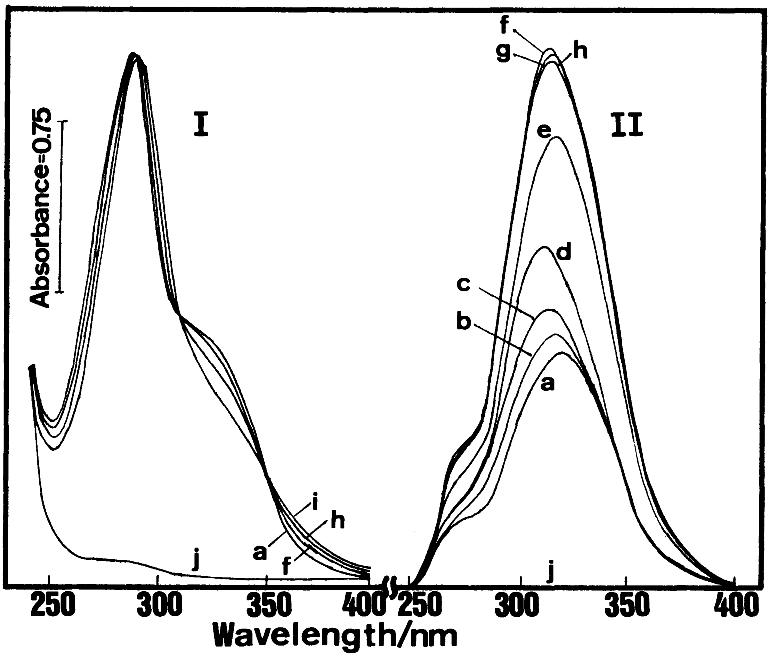

Fig.3. Changes in absorption spectra before the chemiluminescence reaction(I) and the excitation spectra(II) of the total reaction products against additive β-CD concentration, mol·dm^{-3}. a=0;b=1.0×10^{-5};c=2.0×10^{-5};d=5.0×10^{-5};e=7.0×10^{-5}; f=1.0×10^{-4} ;g=5.0×10^{-4} ;h=1.0×10^{-3} ;i=4.0×10^{-3} ; j=1.0×10^{-3} (ABMI not contained); AMBI=1.0×10^{-5} mol·dm^{-3}.

Next, the effects of β-CD on the intermediate course of the CL reaction pathway was estimated. Generally, the relationship between the I_{CL} and ϕ_{fl} of the emitter is given by the following equation.

$$I_{CL} = \phi_{es}\cdot N\cdot\phi_{fl} \quad (\propto \phi_{r}\cdot\phi_{es}\cdot\phi_{fl} = \phi_{CL})^{9)} \qquad (2)$$

N and ϕ_{CL} are the number of ABMI molecules reacted resulting in the critical intermediate ($N \propto \phi_r$), and the overall CL quantum yield, respectively. The dependency of the product $\phi_{es}\cdot N$ for varying β-CD concentration is illustrated in Fig. 4. This figure indicates that β-CD influences the product $\phi_{es}\cdot N$ rather than ϕ_{fl}. Assuming that N is always constant since the CL compound is only ABMI in all systems, it could be speculated that β-CD especially influences ϕ_{es}.

In the luminol system, the emitter is formed from the endoperoxy azo compound, on which most informed speculation is centered, as the key reaction intermediate,10) following the intramolecular chemically initiated electron-exchange luminescence (CIEEL) mechanism.11) Such an intramolecular CIEEL also seems to apply to the ABMI system. On the other hand, Goto et al. reported the significance of the conformation between the electron donor group and the electron acceptor group in the intramolecular CIEEL of the CL of the dioxetanes.12) Taking into account the intramolecular CIEEL, it seems that one of the main possible effects of β-CD on the increment of Φ_{es} is to form a suitable conformation for the intramolecular CIEEL owing to the conformational effects of β-CD, because the intermediate, which is one kind of hydroperoxide, seems to be able to form the inclusion compound with β-CD$^{13)}$ for the suitable conformation. It is, however, difficult to say that the effect of β-CD on Φ_{es} is lost when increasing the β-CD concentration.

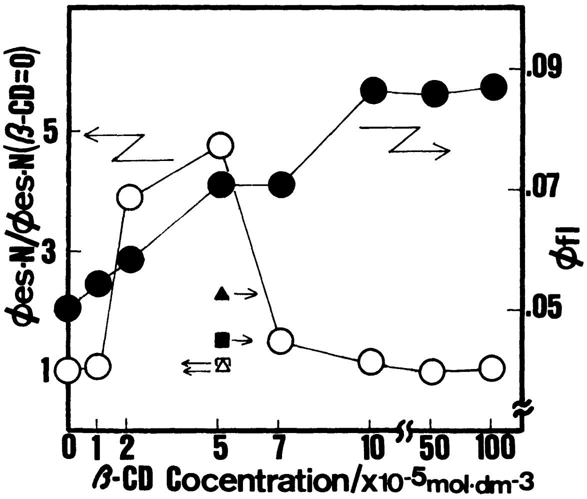

Fig.4. Relationship between the product $\Phi_{es} \cdot N, \Phi_{fl}$ and β-CD concentration Δ :α-CD, □:γ-CD.

Kinetic and NMR studies are now in progress to systematically explain, considering the stabilization effect of β-CD for H_2O_2 also, in both systems with and without a catalyst, such as hemin or peroxidase, using other cyclic hydrazides as well as ABMI. On the other hand, the effects of CD on the enhancement of CL are interesting from the stand point of analytical chemistry, and the applicable study for ABMI as a CL probe utilizing such effects of CD for the determination of the significant biomolecules are also now in progress.

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3) ABMI; MS m/z(%) 262(28), 218(7), 204(100), 191(11); Exact mass calcd for $C_{13}H_{18}O_2N_4$: 262.3007. Found: 262.1415. Found: C, 58.70; H, 6.81; N, 20.89%. Calcd for $C_{13}H_{18}O_2N_4$: C, 59.52; H,6.92; N, 21.36%. The detailed chemiluminescence characteristics and the synthesis of ABMI and the other ABI derivaties will be shortly submitted in another article.

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