April, 1984] **NOTES** *Bull. Chem. Soc. Jpn., 57, 1143—1144 (1984)*

Effect of Ethanol on the Inclusion Complex Formation between *β*-Cyclodextrin and Pyrene

Akira Nakajima

Department of Electronic Engineering, Faculty of Engineering, Saitama Institute of Technology, Okabe, Saitama 369-02

(Received June 2, 1983)

Synopsis. The effect of the addition of ethanol on the formation of the inclusion compound between *β*-cyclodextrin and pyrene in aqueous solution has been studied. Based on the time dependences of the fluorescence spectra of pyrene as a probe, the rate constants estimated for the inclusion and further association in the presence of ethanol have been discussed.

It has been reported[1)] that macromolecular inclusion compounds have a considerable effect on the reactivity of the substrates included. Much interest has recently been shown in the inclusion compounds of cyclodextrins,[2,3)] since they exhibit appreciable enzyme-like activity, and a variety of practical applications are extending. It seems that few studies have been made on the effect of a third component, though it may occasionally have a significant influence on the enzymatic reactions.

Previously, a system of *β*-cyclodextrin (*β*CD) and pyrene in aqueous solution has been investigated utilizing the intensity enhancement phenomenon[4)] and the association constant has been estimated.[5)] In this work, the effect of ethanol on the rates of inclusion and further association has been studied in the same system.

Experimental

Pyrene was recrystallized and vacuum-sublimed, and *β*CD was purified by repeated recrystallizations from water. Ethanol was dried and fractionally distilled.

The sample solutions were carefully prepared using deionized water from an aqueous solution of pyrene which was well saturated by allowing it to stand in contact with pyrene microcrystals for more than a week. No excimer-like emission nor impurity emission was detected in these solutions. The initial concentration of pyrene, C_p, was estimated to be 2.5×10^{-7} M (1 M=1 mol dm^{-3}) by measuring the low absorbance at 334 nm. The volumetric preparation of a solution and the subsequent measurement required at least two minutes, after which the spectral changes with time were observed.

Fluorescence spectra were recorded at about 13 °C on a Shimadzu 210A spectrophotometer with some minor modifications. The excitation was made employing a combination of a high-intensity monochromator and a Xe-lamp stabilized over the wavelengths (about 15 nm width) for the second peak of the second absorption band of pyrene (319—322 nm).

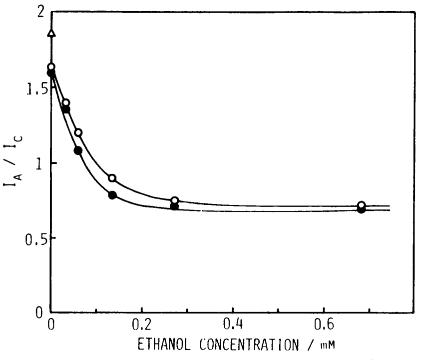

Fig. 1. Changes in I_A/I_C ratios with ethanol concentrations in aqueous solutions of *β*CD (8.5×10^{-4} M) and pyrene (2.5×10^{-7} M). After 30 min (○) and a week (●), and without *β*CD (△).

Results and Discussion

It is now well known[4,6,7)] that the intensities of vibronic bands in the fluorescence spectrum of pyrene vary strikingly with the solvent polarity and the ratio of the intensity of the band A at 373 nm (I_A) to that of the band C at 384 nm (I_C) can be regarded as a measure of the polarity of medium,[6)] the phenomenon being effectively employed here to probe the microenvironments. It is observed that the intensity ratio I_A/I_C is 1.87 in pure water but 0.58 in a nonpolar solvent.

The changes in the I_A/I_C ratios with varying ethanol concentrations in the solutions containing given amounts of pyrene and *β*CD are illustrated in Fig. 1. As can be seen, the effect of the addition of ethanol is very remarkable; the reaction is considerably speeded at concentrations more than 0.2 M. The ratios are also found to change with time.

The decrease of the I_A/I_C value from 1.87[8)] implies that considerable part of pyrene dissolved is included within the *β*CD cavity, where the environment has a polarity corresponding to an I_A/I_C value of 0.68.[5)]

The variations of the I_A/I_C ratios with time for various ethanol concentrations are shown in Fig. 2. Inspection of the behaviors of these curves reveals that they consist of two portions, associated with a fast and a slow process. The fast initial decay, which appears to be almost completed within a minute, is likely due to the inclusion of pyrene by *β*CD, while the subsequent slow decrease may be ascribed to a

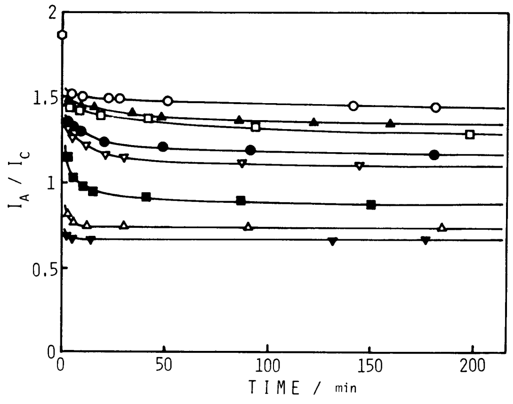

Fig. 2. Variations of I_A/I_C ratios with time in aqueous solutions of *β*CD (1.9×10^{-3} M) and pyrene (2.5×10^{-7} M). Concentration of ethanol (M): 0 (○), 1.4×10^{-3} (▲), 4.1×10^{-3} (□), 8.2×10^{-3} (●), 1.4×10^{-2} (▽), 4.1×10^{-2} (■), 0.14 (△), 1.7 (▼), and without *β*CD (ọ).

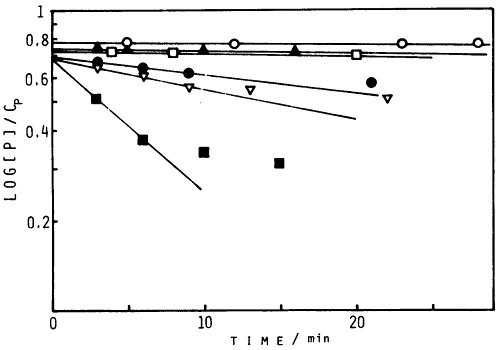

Fig. 3. Plot of log $[P]/C_P$ *vs.* t.

further association of the resulting inclusion compounds. As clearly shown in Fig. 2, the inclusion and the further association are appreciably accelerated by the addition of ethanol.

According to these observations, the reaction scheme may be expressed as follows:

$$P + D \underset{k_2}{\overset{k_1}{\rightleftarrows}} PD,$$

$$PD + D \underset{k_4}{\overset{k_3}{\rightleftarrows}} PD_2,$$

where P and D denote pyrene and βCD, respectively, and PD and PD_2 represent respectively the 1 : 1 and 1 : 2 complexes formed from them. Though the association may produce various complex species,[8] here PD_2 is taken as most probable. These rate constants are considered as functions of ethanol concentration, [Et].

Thus, the kinetic equations are given by

$$\frac{d[P]}{dt} = -k_1[P][D] + k_2[PD],$$

$$\frac{d[PD]}{dt} = k_1[P][D] - (k_2+k_3[D])[PD] + k_4[PD_2].$$

At relatively short times the term associated with $[PD_2]$ can be neglected and [D] is practically equal to C_D, the total βCD concentration, because of $[P] \ll C_D$.

Then, the solution has the form:

$$[P] = A\exp(-k_+t) + B\exp(-k_-t),$$

where

$$k_\pm = (a \pm \sqrt{a^2-4b})/2,$$

$$a = (k_1+k_3)C_D + k_2, \quad b = k_1k_3C_D^2.$$

By using the initial conditions that $[P]=C_P$ and $[PD]=0$ at $t=0$,

$$A = (k_1C_D-k_-)C_P/(k_+-k_-),$$

$$B = (k_+-k_1C_D)C_P/(k_+-k_-).$$

It can be assumed here that $k_1C_D \gg k_2 \approx k_3C_D \gg k_4$.

Accordingly, a rough approximation gives

$$k_+ \approx k_1C_D, \quad k_- \approx k_3C_D,$$

$$A \approx C_P, \quad B \approx k_2C_P/k_1C_D = C_P/KC_D;$$

$$[P]/C_P \approx \exp(-k_1C_Dt) + \exp(-k_3C_Dt)/KC_D.$$

Thus, the rate constant k_3 together with the equilibrium constant K can be estimated from the slowly decreasing portion of the curves. In the present experiment, however, the value of k_1 cannot be obtained owing to the considerably rapid changes.

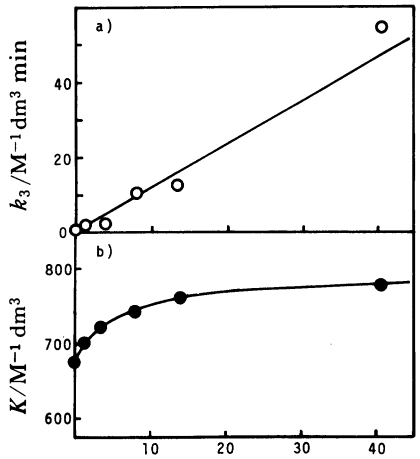

Fig. 4. Plots of k_3 (a) and K (b) against ethanol concentration.

To interpret the time-dependent behaviors on the basis of the above scheme, the concentrations of pyrene dissolved in the water phase, [P], need to be evaluated, and the following formula[5] has been employed:

$$\frac{I_A}{I_C} = \frac{k_P^A\phi_P\varepsilon_P[P] + k_{PD}^A\phi_{PD}\varepsilon_{PD}[PD]}{k_P^C\phi_P\varepsilon_P[P] + k_{PD}^C\phi_{PD}\varepsilon_{PD}[PD]},$$

Where ϕ's are the quantum yields ($\phi_{PD}/\phi_P \approx 1.5$),[5] ε's are the absorption coefficients ($\varepsilon_{PD}/\varepsilon_P \approx 1$),[6] and k^A or k^C represents the fraction of the spectral intensity for the A or C band. The assumption that $[P]+[PD] \approx C_P$ at $t \approx 0$ has been used.

Figure 3 shows plots of log $[P]/C_P$ *vs.* t, which are found to be fairly linear at small t. From the slopes and intercepts of the lines approximate values of k_3 and K are obtained. Furthermore, k_3 as well as K is plotted against the ethanol concentration in Fig. 4. As shown, k_3 appears to depend linearly on [Et], implying the possibility of the formation of a four-component complex PD_2Et.

In constrast to k_3, K does not change so much with [Et] since not only k_1 but k_2 depends on [Et]. Only an order-of-magnitude agreement is obtained between the value of K (675 M^{-1}dm^3) and that (190 M^{-1}dm^3) estimated previously,[5] and the discrepancy may be due largely to the times for measurement, various approximations, different pyrene and βCD concentrations, temperatures, *etc.*

References

1) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York (1975).

2) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry," Springer-Verlag, Berlin (1978).

3) W. Saenger, *Angew. Chem. Int. Ed. Engl.*, **19**, 344 (1980).

4) A. Nakajima, *Kagaku No Ryoiki*, **33**, 49 (1979).

5) A. Nakajima, *Spectrochim. Acta*, **39A**, 913 (1983).

6) A. Nakajima, *Bull. Chem. Soc. Jpn.*, **44**, 3272 (1971).

7) K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.* **99**, 2039 (1977).

8) H. E. Edwards and J. K. Thomas, *Carbohydr. Res.*, **65**, 173 (1978).