Journal of Inclusion Phenomena **4** (1986), 177–183.

Effects of Cyclodextrins on the Fluorescence of Europium Ion–Diketone Complexes

JUHA-PEKKA HIMANEN and TIMO KORPELA
Department of Biochemistry, University of Turku, SF-20500 Turku, Finland

(Received: 2 October 1985; in final form: 7 January 1986)

Abstract. The thermodynamics of the complexation of cyclodextrins (CDs) to naphthyl, phenyl and thienyl subtituted 1,3-diketones, used as sensitizers in time-resolved fluorescence analysis of lanthanides, was studied by gel chromatography. The complexation occurs predominantly at the aromatic end of the diketones with a strength comparable to related aromatic compounds. The effects of CDs on the fluorescence of an europium (III) ion with diketones in an aqueous solution were tested for their applicability in time-resolved fluorescence analysis.

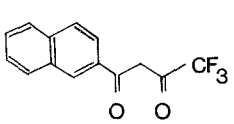
Key words: Time-resolved fluorescence, analysis of lanthanides, europium, cyclodextrins, inclusion compounds.

1. Introduction

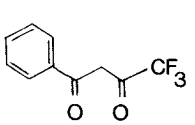
In the course of the development of fluorescence methods and instruments, indications have appeared of replacing radioactive labels by fluorescing ones in routine laboratory applications [1,2]. The most advanced analysis systems utilize delayed fluorescence from an europium ion chelated to diketones in an aqueous micellar solution containing Triton X-100 and trioctylphosphine oxide [2].

It is known that the fluorescence intensity and emission maxima of many fluorescence probes change when they complex to cyclodextrins (CDs; ref. [3]). This has been ascribed as being due to an enhanced rigidity of the phosphor and to simultaneously diminished interactions with quenchers [4]. The spectroscopic properties of the complexed guest molecules resemble those of the compounds when dissolved in dioxane [5]. The chemical environment in the cavity of CDs may thus resemble a micellar solution of the fluorescing agents and, in fact, a simple addition of a heavy-metal-containing component to the CD/lumiphor solution has been claimed to serve as an analytical measuring system [6].

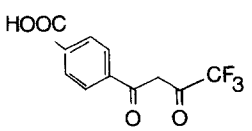
The purpose of the present study was to measure the complexation of CDs with various diketones (shown below) which can be used to transfer absorbed light energy to europium, as well as to characterize the effects of CDs on the system.



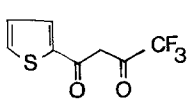
NTA: 2-naphthoyl-trifluoroacetone



BTA: benzoyl-trifluoroacetone



CBTA: *p*-carboxy-benzoyltrifluoro-acetone



TTA: thienoyltri-fluoroacetone

Scheme (**1**)

2. Experimental

2.1. MATERIALS

The cyclodextrins and benzoyltrifluoroacetone (BTA) were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Thienoyltrifluoroacetone (TTA) and trioctylphosphine oxide were obtained from E. Merck (Darmstadt, F.R.G.). 4-Carboxybenzoyltrifluoroacetone (CBTA), 2-naphthoyltrifluoroacetone (NTA), europium (III) chloride and the 'enhancement solution' containing 0.015 mM NTA, 0.05 mM trioctylphosphine oxide and 0.1% Triton X-100 in 0.1 M acetate buffer (pH 3.2) were generous gifts from Wallac (Turku, Finland). Distilled and deionized water was used throughout.

2.2. INSTRUMENTS

The fluorescence spectra were recorded with a Perkin-Elmer MPF-2A fluorescence spectrophotometer. The time-resolved measurements were carried out with a Perkin-Elmer LS-5 luminescence spectrometer.

2.3. METHODS

The equilibrium constants of diketones were essentially determined as described previously [7] by using the Hummel-Dreyer method [8]. A 27 ml column of Sephadex G-25 gel equipped with a water jacket was equilibrated thoroughly with a solution consisting of 0.010 mM diketone in 10 mM acetate buffer, pH 4.3, and the outlet of the column was continuously monitored at 254 nm. The flow rate was maintained at about 25 ml/h and samples of 1.0 ml of 10 mM CD were applied after suitable time periods.

Complexation of α- and β-CD with europium was studied on Biogel P-2 gel onto which α- or β-CDs were immobilized [9]. The runs were performed at 25°C using 0.1 M sodium acetate, pH 3.7, supplemented with 0.5 M NaCl as the eluent and fractions collected and analyzed for by their fluorescence.

The fluorescence spectra were recorded at about 23°C in 100 mM acetate, pH 3.7. The concentration of europium was usually 0.01 mM except in the time-resolved experiments where it was 0.001 mM. The concentrations of trioctylphosphine oxide and diketones were usually 0.015 and 0.05 mM, respectively.

3. Results and Discussion

3.1. THERMODYNAMICS OF COMPLEXATION OF DIKETONES WITH CDs

Preliminary solubilization tests of diketones by CDs clearly showed some complex formation, since a typical microemulsion of diketones in aqueous solution disappeared when CDs were added. The equilibrium values were determined by gel chromatography at various temperatures [7] in very low concentrations of diketones. The equilibrium constants varied between 15 and 1300 M^{-1} at 25°C (Table I), β-CD complexing most effectively.

Assuming that it is the aromatic moiety of the diketones which causes the interaction with CDs, complexes of β-CD with, e.g., β-naphthol, benzoic acid, and benzoyl acetic acid can serve as model compounds. Their equilibrium constants are 625 [10], 610 [11] and 102 M^{-1} [12], respectively, values which are in reasonable accordance with those shown in Table I and

Table I. Thermodynamic values for complexation of various diketones with CD's in 10 mM acetate buffer (pH 4.3)

Diketone	CD	$K_{298\,K}$ (M^{-1})	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
	α	73.1			
NTA	β	1320	− 17.8	− 37.8	− 67.1
	γ	142			
	α	< 15			
CBTA	β	237	− 13.5	− 17.8	− 14.4
	γ	26.8			
	α	35.6			
TTA	β	216	− 13.3	− 12.8	+ 1.68
	γ	–			
	α	39.7			
BTA	β	418	− 15.0	− 15.4	− 1.34
	γ	157			

thus indicate that the diketone function does not significantly contribute to the stabilization of the complex, although some hydrogen bonding might have been expected. In the case of CBTA, the ionized form was studied. Its equilibrium constant with α-CD was below 15 M^{-1} (Table I). The *K*-value of benzoic acid with α-CD decreases from 722 M^{-1} to 11.2 M^{-1} when the guest molecule deprotonates [13].

The classical hydrophobic interaction has generally been considered to be associated with a favourable change in entropy, whereas the stabilization by dipolar forces refers to a large negative change in enthalpy [14]. The diketones (Table I) seem to complex mainly through dipolar forces because the contribution of the entropy term is unfavourable (NTA and CBTA)

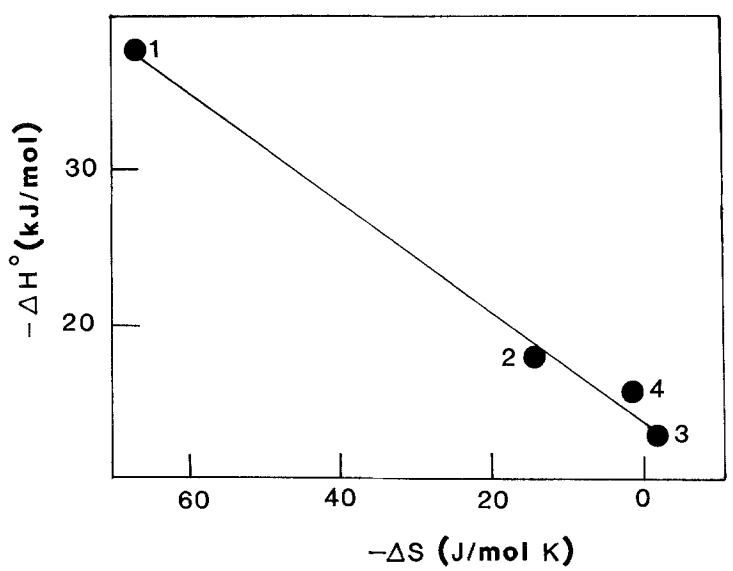


Fig. 1. A plot of $\Delta H°$ *vs.* $\Delta S°$ values for binding of various diketones to β-CD. Abbreviations: 1 = NTA, 2 = CBTA, 3 = TTA, 4 = BTA.

or negligible (TTA and BTA). The more favourable entropies of TTA and BTA can be attributed to the relatively small sizes of the molecules and to the freedoms of molecular motions inside the CD cavity [15]. The structures of CBTA and BTA differ only in respect of one carboxylate in the *para*-position of the phenyl ring. The enthalpy term of CBTA is larger but it is compensated by an unfavourable entropy indicating a limited solvation of carboxylate inside the CD cavity.

Figure 1 shows a plot of ΔH° *vs.* ΔS° values drawn from Table I. The points fall on one line with a correlation coefficient of 0.997. Thermodynamic parameters, measured in related conditions for *para*-nitrophenol [7], which is known to form 1 : 1 complexes with CDs, also smoothly fitted the line. The linear correlation should be considered to indicate the absence of a ternary complexation mode [16]. In conclusion, it is evident that 1 : 1 adducts are formed and the complexation takes place at the aromatic part of the diketones with a strength comparable to that shown by related aromatic compounds.

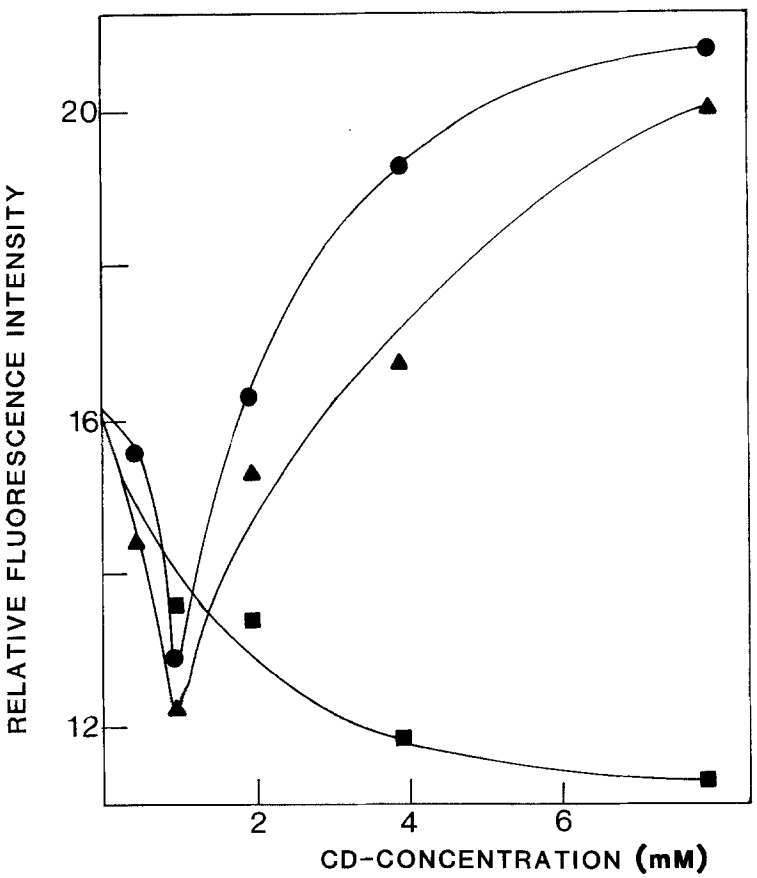


Fig. 2. Effect of CDs on the relative fluorescence intensity of a solution containing 0.5 mM NTA, 0.15 mM trioctylphosphine oxide and 0.01 mM europium in 100 mM acetate buffer, pH 3.7. Symbols: ■ = α-CD, ● = β-CD, ▲ = γ-CD.

3.2. EFFECTS OF CDs ON SOLUTIONS CONTAINING EUROPIUM AND DIKETONES

When CDs were added to aqueous solutions containing diketone and europium (III) chloride, they all decreased the fluorescence intensity. The initial level of fluorescence was very low compared to the respective values in organic or micellar solutions. The addition of trioctylphosphine oxide in a 1.5-fold molar excess of europium caused a 3-fold increase in the fluorescence intensity. Since this agent has often been exploited in related systems [2], it was used in later experiments.

Figure 2 shows the effect of CD concentration on the fluorescence of an aqueous mixture containing 0.5 mM NTA, 0.15 mM trioctylphosphine oxide and 0.01 mM europium. All three CD forms first decreased the europium fluorescence but with β- and γ-CDs there was a sharp inflection point at about 1 mM CD, thereafter the fluorescence increased above the initial level. The fluorescence at 8 mM CD did not change as a function of time. When the concentrations of diketones and trioctylphosphine oxide were reduced to one tenth, all CD forms smoothly decreased the fluorescence intensities with each of the diketones. The equilibrium constants between α- or β-CD and europium were measured by a chromatographic method (see Experimental) to be below 5 M^{-1}, which indicates that direct complexation of europium was not involved. Based on the above observations, we explain the results of Figure 2 as follows. In the experiment the concentration of NTA was high and, hence, it mainly existed as molecular aggregates or micelles together with trioctylphosphine oxide. The addition of CDs led to a rapid disintegration of these structures aggravating the complexation of europium to diketones while in high β- or γ-CD concentrations NTA was totally solubilized, possibly by a coinclusion with trioctylphosphine oxide, especially in the case of the γ-form. The final condition was approximately the same as if a high concentration of monomeric NTA had been dissolved in a solution. It is apparent from Table I that NTA complexes poorly with α-CD. Instead, it is presumed that the α-form can include the alkyl groups of trioctylphosphine oxide (17) and the effect of α-CD in Figure 2 can thus be explained.

The fluorescence of europium (0.01 mM) was studied in a solution containing 0.05 mM diketone, 0.015 mM trioctylphosphine oxide, and CDs. In each of the diketone solutions the CDs usually decreased the fluorescence in accordance to the numerical values of the equilibrium constants in Table I; i.e., the order of the decrease was $\beta > \gamma > \alpha$. The most drastic decrease in the intensity was found in the system of β-CD with CBTA in which a fluorescence of a few percent of the original value was obtained in 5 mM CD. Because this diketone contains a carboxylate group, it is presumed that the presence of the ionized group resulted in the CD species including the polar diketone functions of CBTA thus making them inaccessible to europium.

In the above-described experiments CDs were added last in the measuring cuvette. Whenever diketones were added in the place of CDs, different kinds of results were obtained: when 5 mM β- or γ-CD were used, the fluorescence intensity of NTA and CBTA solutions clearly rose above the non-CD level whereas α-CD acted as previously (Figure 2). The normal situation was, however, attained after $\frac{1}{2}$–1 h. When diketones were added last, different adducts were probably generated in the solution. These structures reorganized to the energetically most favourable ones very slowly in contrast to those formed via the prior addition of diketones.

3.3. TIME-RESOLVED FLUORESCENCE MEASUREMENTS

It was desirable to further study the possible origin of the lowered fluorescence intensity caused by CDs. For this purpose a system containing 0.05 mM CBTA, 0.015 mM trioctylphosphine oxide and 0.001 mM europium ion in the usual buffer solution supplemented with 5 mM β-CD was preliminary studied by time-resolved fluorometry. The plots applied to calculate the time constants ($1/\tau$) of the fluorescence proved to be nonlinear referring to two or more molecular species in a different chemical surrounding, whether CD was added or not (18) and, hence, no exact values can be given. However, qualitative time constants for CD-containing solutions were only one-half or less of the values obtained without CD. This means that while CD drastically diminishes the fluorescence intensity, the duration of the remaining fluorescence increases at the same time. The latter comes from europium ions, which are considerably shielded from the bulk solution but which represent only a relatively minor population of all the metal ions. The main part of the ions are probably either unable to chelate with diketones or are coimmobilized into the CD-cavity in such a way that they are sterically far from the diketone functions.

3.4. FLUORESCENCE FROM EUROPIUM IN TRITON X-100 SOLUTION

When nonionic detergents, like Triton X-100, are added to the solution containing NTA, trioctylphosphine oxide and europium ion, the quantum yield from europium is greatly enchanced due to a transfer of the fluorescing system into organic solution [2]. A commercial

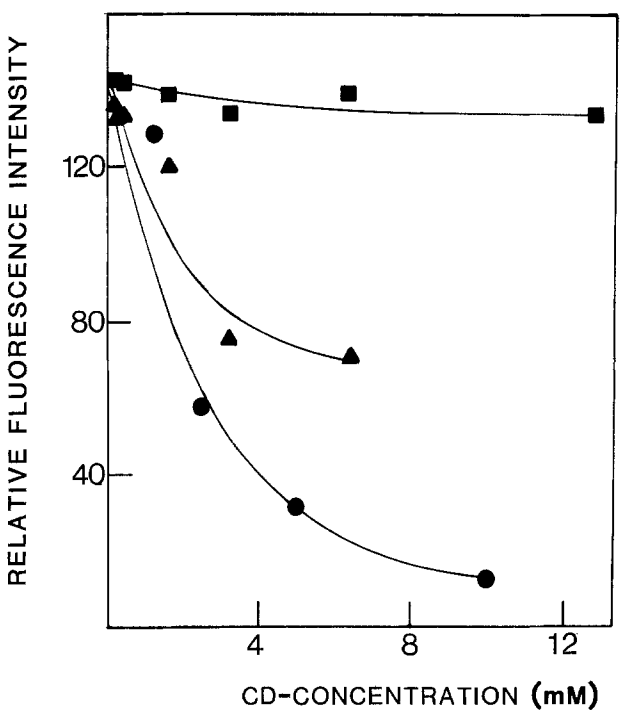


Fig. 3. Effect of CDs on the relative fluorescence intensity of 0.01mM europium measured by the 'enhancement solution', commercially available from Wallac Oy, Turku, Finland. Symbols: ■ = α-CD, ● = β-CD, ▲ = γ-CD.

reagent for the quantitation of europium containing highly purified components is now available (Wallac OY, Turku, Finland). It can be presumed that the added europium will be solubilized on the outer surfaces of the micelles [19], possibly by means of the polar heads of the diketone. The addition of CDs to the solution might alter the outer shell of the micellar structures to a more favourable direction for the fluorescence. Figure 3 shows the effects of different CD species on the fluorescence of 0.01 mM europium measured by Wallac's 'enhancement solution'. The results of Figure 3 parallel those obtained without detergent (see experiments above). The decrease in fluorescence was largest with β-CD while α-CD had practically no effect although it can be assumed to complex with both trioctylphosphine oxide and Triton X-100 molecules. The effect of β- and γ-CDs can be ascribed to a removal of NTA out of the micelles into the aqueous phase.

In conclusion, in spite of occasional slight positive effects of CDs on the fluorescence of the europium-diketone system, they do not seem to have any real analytical advantages in this system. With other fluorescence probes they might, however, have some use, but unfortunately such systems are likely to be difficult to design with methods other than trial and error.

Acknowledgements

We would like to thank Jouko Kankare for the advice concerning the time-resolved fluorescence measurements and Ilkka Hemmilä and Timo Lövgren for helpful discussions. The work was financially supported by LKB-Wallac (Turku, Finland) which is greatly appreciated.

References

1. E. Soini and H. Kojola: *Clin. Chem.* **29**, 65 (1983).
2. I Hemmilä, S. Dakubu, V.-M. Mukkala, H. Siitari, and T. Lövgren: *Anal. Biochem.* **137**, 335 (1984).
3. F. Cramer, W. Saenger, and H.-Ch. Spatz: *J. Am. Chem. Soc.* **89**, 14 (1967).
4. N. J. Turro, J. D. Bolt, Y. Kuroda, and I. Tabushi: *Photochem. Photobiol.* **35**, 69 (1982).
5. R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender: *J. Am. Chem. Soc.* **89**, 3242 (1967).
6. S. Scypinski and L. J. Cline Love: *International Laboratory*, April 1984, p. 60.
7. T. K. Korpela and J.-P. Himanen: *J. Chromatogr.* **290**, 351 (1984).
8. J. P. Hummel and W. J. Dreyer: *Biochim. Biophys. Acta* **63**, 530 (1962).
9. J.-P. Himanen, A. Elo, and T. K. Korpela in *Chemical and Biological Aspects of Vitamin* B_6 *Catalysis* (Ed. A. Evangelopoulos), Part A, p. 109, Alan R. Liss, New York (1984).
10. T. Yorozu, M. Hoshino, M. Imamura and H. Shizuka: *J. Phys. Chem.* **86**, 4422 (1982).
11. R. I. Gelb, L. M. Schwartz, M. Radeos, and D. A. Laufer: *J. Phys. Chem.* **87**, 3349 (1983).
12. T. S. Straub and M. L. Bender: *J. Am. Chem. Soc.* **94**, 8881 (1972).
13. K. A. Connors, S.-F. Lin, and A. B. Wong: *J. Pharm. Sci.* **71**, 217 (1982).
14. J. C. Harrison and M. R. Eftink: *Biopolymers* **21**, 1153 (1982).
15. S. Hamai: *Bull. Chem. Soc. Jpn.* **55**, 2721 (1982).
16. R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson, and D. A. Laufer: *J. Am. Chem. Soc.* **103**, 1750 (1981).
17. W. P. Jencks: *Catalysis in Chemistry and Enzymology*, p. 409, McGraw-Hill, New York (1969).
18. N. J. Turro, T. Okubo, and C.-J. Chung: *J. Am. Chem. Soc.* **104**, 1789 (1982).
19. J. H. Fendler and E. J. Fendler: *Catalysis in Micellar and Macromolecular Systems*, p. 45, Academic Press, New York (1975).