Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **11**: 313–322, 1991.

Investigation of Induced Circular Dichroism of Benzo(*a*)pyrene Cyclodextrin Complexes

GABOR PATONAY
Department of Chemistry, Georgia State University, Atlanta, GA 30303, U.S.A.

ISIAH M. WARNER*
Department of Chemistry, Emory University, Atlanta, GA 30322, U.S.A.

(Received: 30 October 1990; in final form: 5 April 1991)

Abstract. This study focuses on the inclusion complexes of α-, β- and γ-cyclodextrins with benzo (*a*) pyrene, as indicated by induced circular dichroism data. The benzo(*a*)pyrene complex exhibits a significant induced ellipticity in the presence of γ-cyclodextrin, while the other two cyclodextrins did not produce a significant induced circular dichroism signal. In the presence of deuterium oxide, which is larger than the water molecule, the ellipticity is larger. The changes in ellipticity with increasing cyclodextrin concentration are observed to follow the changes in equilibrium of the pyrene–γ-cyclodextrin system. The study of these systems using circular dichroism measurements yields a method of estimating the possible stoichiometry of this complex.

Key words. Complex formation, induced circular dichroism.

1. Introduction

In recent years, there has been considerable interest in the inclusion compounds of cyclodextrins (cycloamyloses) with polynuclear aromatic hydrocarbons (PAHs). Cyclodextrins (CYDs) are torus shaped cyclic oligosaccharides with the ability to act as host for selected guest molecules. These guest/host properties are largely attributed to the ability of cyclodextrin molecules to form different guest–host association complexes. Although the 1 : 1 guest–host complex is most common, 2 : 1, 2 : 2 or 1 : 2 complexes have also been reported in the literature [1–3]. The most frequently studied cyclodextrins are of the α, β and γ types with cavity diameters that favorably complex with PAH molecules of different sizes. The cavities of these cyclodextrins are relatively hydrophobic, providing a favorable microenvironment for apolar guests such as PAHs.

The spectral properties of the complexed guest molecule is often markedly different from the non-complexed species. Hence, spectral measurements are frequently used to characterize cyclodextrin complexes. Although spectral measurements can supply a wealth of information for characterization of various complexes, such information is often not suitable for investigation of these complex systems. The formation constant of these host–guest complexes are often too small to be evaluated by less sensitive spectral measurements such as circular dichroism (CD). References to induced circular dichroism of cyclodextrin complexed

* Author for correspondence.

fluorophores date back to as early as 1979. The earliest studies investigated induced CD of substituted benzenes in the β-cyclodextrin cavity [1, 4]. The induced CD of azo dyes and similar dyestuffs have also been thoroughly investigated [5, 6]. Some rather simple rules have been formulated for describing induced CD of aromatic guest molecules in cyclodextrin hosts [7]. It has also been shown that the inclusion of two or more guest molecules can often be observed with induced CD [2]. However, the interaction with one guest molecule can also induce chirality in the chromophore system of the guest molecule [8]. The presence of induced CD peaks has been observed for the complexation of achiral drugs with different cyclodextrins [9].

A large number of studies have examined cyclodextrin (CYD) complexes and the ability of CYDs to enhance fluorescence and phosphorescence of selected fluorophores [13, 14]. This enhancement phenomenon of cyclodextrins is mainly ascribed to compartmentalization and shielding of the excited singlet and triplet species of the fluorophore. Several studies [10–12] have provided detailed information on the induced CD effect for pyrene–cyclodextrin complexes. Although pyrene is an environmentally important PAH compound, it is less significant with respect to toxicity and carcinogenicity as compared to other PAHs, such as benzo(*a*)pyrene (BAP). However, there has not been much research on the cyclodextrin complexes of BAP.

Other phenomena indicate the formation of more complicated complexes [15, 16]. Pyrene has been an excellent probe for investigating such phenomena. For example, the enhancement of pyrene excimer fluorescence is a good indication of the presence of two guest molecules in the cavity of one CYD molecule [17]. It is more difficult to deduce the formation of 1 : 2 complexes from absorbance data which is related to the ground state of the complexed fluorophore. The presence of induced circular dichroism is one of the indications of such 1 : 2 complexes. However, it should be noted that one can observe induced CD even in the case of 1 : 1 complexes since the CYD molecule itself is asymmetric [13]. Unfortunately, the formation constant of the host–guest complex or the solubility of the guest is often too small for adequate evaluation of these data. This is especially true in the case of the benzo(*a*)pyrene–cyclodextrin system, where the low aqueous solubility of benzo(*a*)pyrene ($\approx 10^{-8}$ M) [18] can introduce a number of problems in spite of the relatively high formation constant of the benzo(*a*)pyrene–CYD complex [19, 20].

A number of spectral methods may be used for the investigation of pyrene–CYD inclusion complexes. Some of these methods have been applied frequently [7, 13, 19]. The application of ternary complex formation [19, 21], time-resolved emission spectroscopy [20], or induced circular dichroism [22] are just a few examples of such studies.

The study described in this paper will focus on the induced CD of benzo(*a*)pyrene when complexed with γ-cyclodextrin. In addition, the formation constant for the equilibrium between benzo(*a*)pyrene and γ-cyclodextrin is evaluated. Pyrene has been used mostly as a probe molecule for studying the microenvironment of the cyclodextrin cavity. Detailed studies about that chemical system make benzo(*a*)pyrene a logical probe for further studying cyclodextrin complexation phenomena and induced CD in the case of formation of cyclodextrin–PAH complexes.

2. Experimental

Benzo(*a*)pyrene (99+% purity) and deuterium oxide (NMR grade) were obtained from Aldrich Chemical Company (Milwaukee, WI), and γ-cyclodextrin was obtained from American Maze (Hammond, IN). All solvents used in this study were ACS grade unless otherwise noted. Stock solutions of benzo(*a*)pyrene were prepared by dissolving benzo(*a*)pyrene in benzene (99+%, thiophene free, Aldrich) to make a 10^{-2} M solution. It was necessary to use benzene as the solvent due to the extremely high concentration required for the stock solution. No other solvent system of low boiling point was found to be suitable for dissolution of benzo(*a*)pyrene. In addition, the use of benzene for preparation of benzo(*a*)pyrene stock solution eliminated the need for removal of large amounts of solvent. Removal of a larger amount of solvent is undesirable because this could result in crystallization of benzo(*a*)pyrene on a relatively large surface area of the inner wall of the vial. By use of this concentrated benzo(*a*)pyrene stock solution, we assured that BAP crystals were deposited on the bottom of each vial covering a relatively reproducible surface area of the glass.

The CYD stock solutions were prepared by dissolving appropriate amounts of CYD in deionized water (Continental Water Systems, GA) or in deuterium oxide to make stock solutions with exactly controlled concentrations of 2–40 mM. The CYD solutions were never stored for more than three days, using refrigeration, in order to minimize interferences from bacterial degradation products. Benzo(*a*)pyrene–CYD complex samples were prepared by pipetting 500 μL of benzo(*a*)pyrene stock solution into a small vial and completely evaporating the solvent (benzene). In this manner, 5×10^{-6} moles of pyrene crystals (1.06 mg) were deposited on the glass wall of the vial used for solution preparation. This method ensured that all samples had the same amount of solid pyrene present and that the crystal structure (size and form of pyrene crystals) were uniform during the studies. Once the solvent was completely removed, the vials were filled with 10 mL of CYD stock solutions of different concentrations. The solutions were equilibrated with the benzo(*a*)pyrene crystals deposited on the inner wall of the vials for 24 hours at room temperature without use of sonication or any other method that could increase the formation of microcrystals.

After equilibration, the solutions were filtered using a 0.05 micron pore size syringe filter (Millipore) to remove excess benzo(*a*)pyrene crystals. This procedure gave samples of CYD concentrations equal to the original CYD stock solutions and benzo(*a*)pyrene concentrations as determined by the equilibrium. This procedure was followed to ensure that all benzo(*a*)pyrene molecules in the samples were solvated in the solvent or in the form of the complex as determined by the equilibrium, and not suspended as microcrystals. This procedure was necessary in order to increase the concentration of the benzo(*a*)pyrene-cyclodextrin complex. When solutions were prepared where benzo(*a*)pyrene concentrations were at or below the aqueous solubility limit, the concentration of the complex was so small that no induced CD effect could be observed in a reproducible manner. These low benzo(*a*)pyrene concentrations are suitable only for more sensitive spectral measurements such as fluorescence. In contrast, the procedure described above utilize the solubilization phenomena of cyclodextrin complexation for increasing the concentration of the complex [23].

Absorption spectra were taken on a Varian DMA 200 spectrophotometer. The concentrations of solutions were too high for observing fluorescence spectra due to inner-filter effects. Dilutions of these solutions for fluorescence measurements would change the equilibrium. Circular dichroism spectra were obtained using a Jasco J-600 spectropolarimeter. The data were taken at 20°C unless otherwise noted. Solutions in equilibrium with air were used. Solutions of the benzo(*a*)pyrene–CYD complex prepared according to the above procedure were used on the day of preparation.

3. Results and Discussion

Representative absorption spectra for the benzo(*a*)pyrene in aqueous 28.64 mM γ-cyclodextrin solution are shown in Figure 1. As shown significant changes can be observed in the spectra of complexed benzo(*a*)pyrene when compared to the uncomplexed chromophore [24]. These changes are expected due to the strong interactions resulting from the relatively 'tight' fit of benzo(*a*)pyrene in the γ-cyclodextrin cavity. Other cyclodextrin concentrations showed similar spectra and therefore only the relative absorbances are reported at three characteristic wavelengths in Table 1. Since no direct method is available for determining the concentration of the complexed benzo(*a*)pyrene due to the lack of information on molar absorptivity values of cyclodextrin–benzo(*a*)pyrene complexes, the equilibrium constant for the complex may be used for an estimate. For our purpose, we

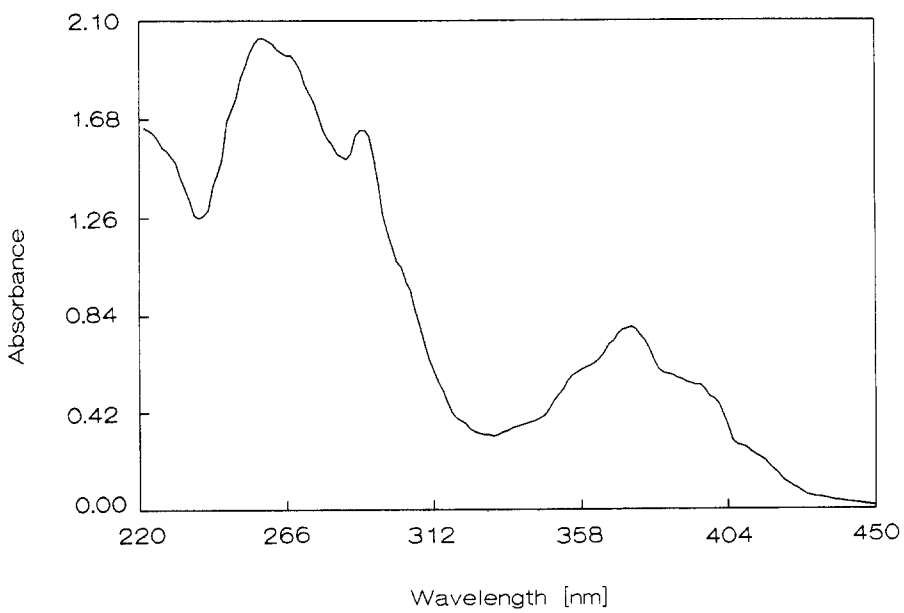

Fig. 1. Representative absorption spectra for benzo(*a*)pyrene in aqueous γ-cyclodextrin solution (28.64 mM).

Table I. Absorption maxima of benzo(*a*)pyrene–γ-cyclodextrin complexes.

γ-CYD Conc. [mM]	A_{258nm}	A_{289nm}	A_{372nm}
40.09	2.64	2.06	1.01
28.64	2.05	1.66	0.793
17.18	1.80	1.43	0.675
11.45	1.22	0.954	0.445
5.73	0.546	0.419	0.190
2.86	0.249	0.188	0.080

can estimate the molar absorptivity of the complexed benzo(*a*)pyrene using the equilibrium constant to determine if the molar absorptivity of the complex is different from that of the uncomplexed benzo(*a*)pyrene. The equilibrium constant of the benzo(*a*)pyrene–γ-cyclodextrin system has been determined in the past [18]. Using this value, we could estimate the concentration of the free and complexed form of the pyrene provided we have information about the stoichiometry of the complex.

Representative induced circular dichroism data of the benzo(*a*)pyrene: γ-cyclodextrin complex are depicted in Figure 2 at four different cyclodextrin concentrations in the region of 10–40 mM. As expected, the measured magnitude of induced chirality is strongly dependent on the concentration of γ-cyclodextrin since increasing cyclodextrin concentration should increase the concentration of the complex as determined by the equilibrium.

Induced CD data may be used to estimate the stoichiometry of the complex. The value of induced CD is determined by

$$[\theta] = k\frac{\Delta E_c}{c} \tag{1}$$

where k is a proportionality constant; $[\theta]$ is the molar ellipticity; ΔE_c is the CD of the complex and c is the concentration of the complex. After rearranging Equation 1, we obtain

$$\Delta E_c = \frac{[\theta]}{k} c \tag{2}$$

indicating that the measured CD (ΔE_c) should be proportional to the concentration of the complex. The concentration of the complex can be calculated from the equilibrium constant:

$$K_{eq} = \frac{[\text{CYD–BAP}]}{[\text{CYD}]\,[\text{BAP}]} \tag{3}$$

where K_{eq} is the formation constant of the cyclodextrin–benzo(*a*)pyrene complex, [CYD–BPY], [CYD] and [BPY] are the respective concentrations of the complex,

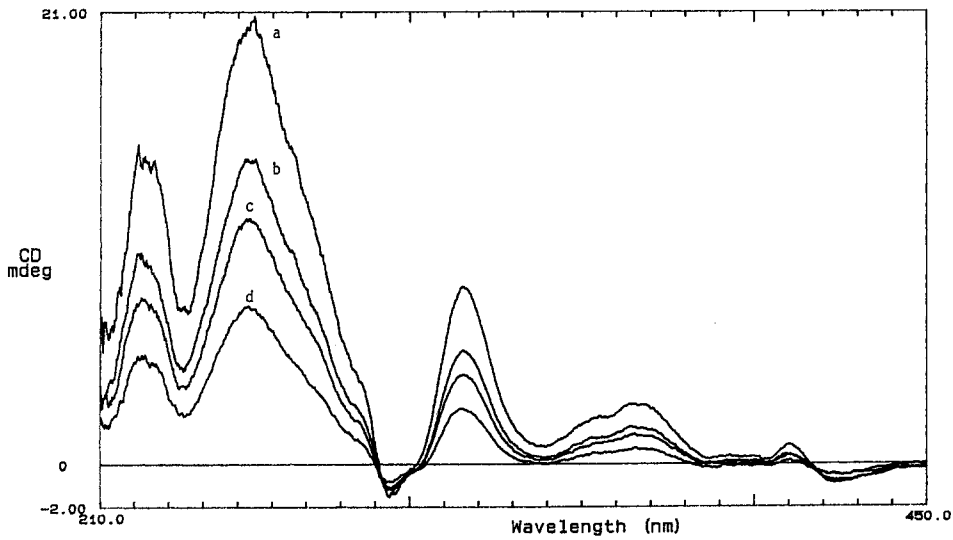

Fig. 2. Representative induced circular dichroism data for the benzo(*a*)pyrene in aqueous γ-cyclodextrin solution at four different cyclodextrin concentrations (a: 40.08 mM, b: 28.64 mM, c: 17.18 mM, d: 11.45 mM).

free cyclodextrin and benzo(*a*)pyrene. In Equation (3), we assumed that the equilibrium corresponds to the formation of a 1 : 1 complex, i.e.:

$$\text{BAP} + \text{CYD} \leftrightarrow \text{CYD–BAP} \tag{4}$$

Since benzo(*a*)pyrene is present in the solid form while it is equilibrated at room temperature, the concentration of its uncomplexed form is constant, i.e. equal to its aqueous solubility. Since the system is allowed to come to equilibrium, this concentration does not change after filtration of the solution. We can express the concentration of the benzo(*a*)pyrene complex using Equation (3). Rearranging this equation, we obtain

$$[\text{CYD–BAP}] = K_{\text{eq}}[\text{CYD}][\text{BAP}] \tag{5}$$

A constant free BAP concentration indicates that the concentration of the complex will be proportional to the concentration of γ-cyclodextrin. This is valid even for 2 : 1 guest–host complexes, where the equilibrium is in the form of

$$2\text{BPY} + \text{CYD} \leftrightarrow \text{CYD–(BPY)}_2. \tag{6}$$

Such would result in the concentration of the complex also being proportional to the concentration of γ-cyclodextrin, i.e.,

$$[\text{CYD–(BPY)}_2] = K_{\text{eq}}[\text{CYD}][\text{BPY}]^2 \tag{7}$$

Using Equations (2) and (5), we obtain

$$\Delta E_{\text{c}} = \frac{[\theta]}{k} K_{\text{eq}}[\text{CYD}][\text{BPY}] \tag{8}$$

If all constant values are combined into one constant, K_{CD}, we obtain the final expression for the induced CD:

$$\Delta E_c = K_{CD}[CYD] \tag{9}$$

indicating that the measured CD plotted against the concentration of free cyclodextrin should produce a linear relationship if a 1 : 1 or 2 : 1 stoichiometry is followed by the system.

The magnitude of the concentration of the complex relative to the free cyclodextrin may be estimated using the initial parameters. As indicated earlier, 5×10^{-6} moles of benzo(*a*)pyrene was used in the solid form to prepare the complex. We observed that most of the crystals remained undissolved indicating that only a small portion of the benzo(*a*)pyrene was dissolved even at the highest γ-cyclodextrin concentrations used in this study (40.09 mM). Even if we assume that all benzo(*a*) pyrene dissolves to form the complex, this would result in only a 5×10^{-4} M solution, a significantly lower value than that of the γ-cyclodextrin.

This indicates that most of the γ-cyclodextrin is not complexed with benzo(*a*) pyrene. Since the concentration of the complex is only a small fraction of that of the free CYD, we can substitute the free [CYD] with the initial analytical concentration of γ-cyclodextrin. If we plot ΔE_c as a function of the analytical concentration of γ-cyclodextrin, we can determine the molar ellipticity of the complex at each wavelength. The results of the plots according to Equation (9) are depicted in Figure 3. Since benzo(*a*)pyrene is present in excess at the time of the preparation of the solutions, the straight line in Figure 3 indicates the formation of either a 1 : 1 or 2 : 1 guest–host complex. If different complexes are formed, we should observe deviations from linearity. For example, if we assume the formation of 1 : 2 or 2 : 2 complexes, no linear relationship would be observed.

In the cases of 1 : 2 or 2 : 2 stoichiometry, the form of Equation (6) would be

$$\Delta E_c = K_{CD}[CYD]^2 \tag{10}$$

However, this form of the ΔE_c function is not supported by our data (Figure 3). Accordingly, we can assume that either a 1 : 1 or a 2 : 1 complex is formed. Nevertheless, the formation of a 1 : 1 complex is more likely if we take the molecular dimensions of the molecules into consideration. The size of the γ-cyclodextrin cavity is not large enough to support the inclusion of two guest molecules. Hence, we can assume the formation of 1 : 1 complexes.

Using the absorptivities of the complex as calculated from the absorption spectra, we can also estimate the molar absorptivity of the complex. The solubility of benzo (*a*) pyrene in water has been determined in the past [18], 5.6×10^{-9} M. Using these data and the formation constant determined earlier from these data [18], we can obtain a concentration value of 1.57×10^{-5} M for the benzo(*a*)pyrene γ-cyclodextrin complex at 40.09 mM γ-cyclodextrin concentration. The molar absorptivity of the complex can be determined from Figure 1 if the concentration of the complex is known. Using these data we obtain $\varepsilon = 177\,000$ for the molar absorptivity of the complex at the maximum absorption wavelength, indicating a higher molar absorptivity for the complex than for the free benzo(*a*)pyrene.

No induced CD effect or increased solubilization of benzo(*a*)pyrene as measured by absorption spectra was observed for α-cyclodextrin. Several concentrations of

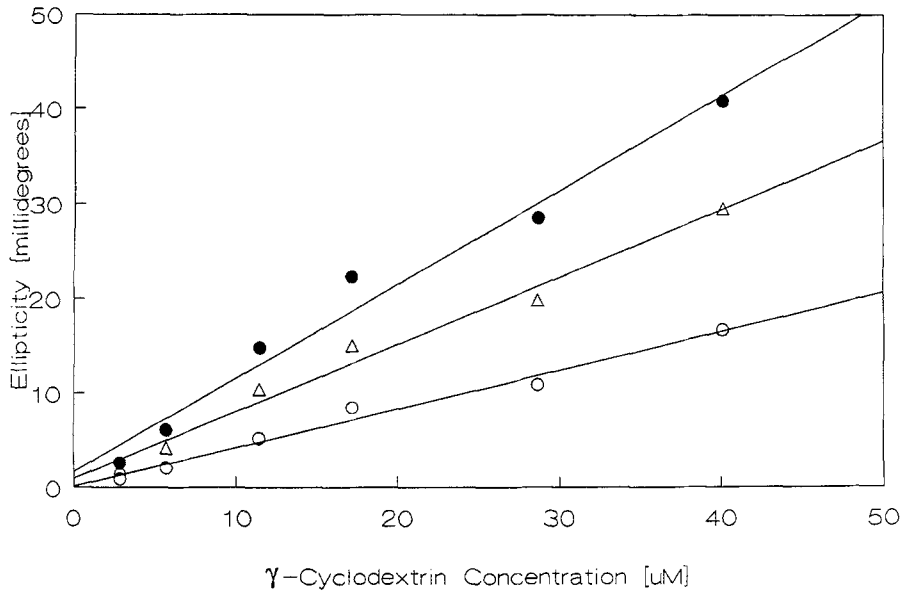

Fig. 3. Induced circular dichroism of benzo(*a*)pyrene–γ-cyclodextrin complex as a function of cyclodextrin concentration (○: 253 nm, △: 222 nm, ○: 314 nm).

α-cyclodextrin solutions were tested up to 50 mM without the indication of induced CD. Considering the size of the α-cyclodextrin cavity, this observation seems to be appropriate. Similarly, a negligible effect was observed when the formation of β-cyclodextrin benzo(*a*)pyrene complexes were studied. Although the formation constant of this complex is larger than that of the α-CYD-BYP complex [18], it is still significantly lower than that of the γ-CYD-BYP complex. Further difficulties are introduced by the low solubility of β-cyclodextrin preventing the preparation of the high concentrations of CYD needed for the methodology described in this paper.

It is likely that water molecules play an important role in the complexation. This possibility may be investigated if deuterium oxide is used as solvent in our experiments. When deuterium oxide is substituted for water while other experimental conditions are kept unchanged, the magnitude of the induced circular dichroism is larger, but the spectra are essentially the same. This is illustrated in Figure 4, where induced circular dichroism spectra are seen for the benzo(*a*)pyrene γ-cyclodextrin complex in deuterium oxide. Figure 4 depicts the induced CD effects for the complex formation under the exact same experimental conditions as in Figure 2 for curve a, i.e. at 40.09 mM γ-cyclodextrin concentration. The larger molecular size of deuterium oxide probably plays an important role in this phenomena.

4. Conclusion

In summary, it can be concluded that induced circular dichroism, especially when combined with other spectroscopic methods can provide valuable information about the nature of cyclodextrin complexation as well as the possible stoichiometry

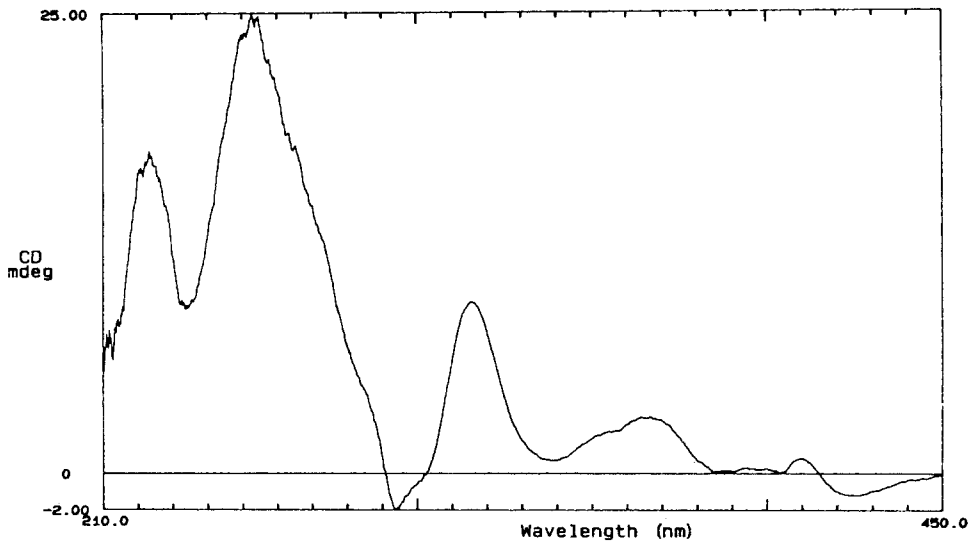

Fig. 4. Representative induced circular dichroism spectra for the benzo(*a*)pyrene–γ-cyclodextrin complex in deuterium oxide obtained under exactly the same conditions as in Figure 2a.

of the complex. In fact, these results show that it is often necessary to investigate chirality to understand these complex systems. Neither α- nor β-cyclodextrin could induce significant chirality into this large polynuclear aromatic compound. The stoichiometry of the complex with γ-cyclodextrin is suggested to be a 1 : 1 guest–host stoichiometry. Due to the higher formation constant between γ-cyclodextrin and benzo(*a*)pyrene and the tight fit inside of the cyclodextrin cavity, significant chirality is induced in the achiral benzo(*a*)pyrene molecule. Further studies are necessary to evaluate the effect of third components, e.g. alcohols, on the magnitude of induced chirality and on the evaluation of other characteristics of the complex, e.g., formation constants using only circular dichroism data.

Acknowledgement

This work was supported in part by the National Science Foundation (CHE-9001412) and the National Institutes of Health (GM 39844). G.P. also acknowledges the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research.

References

1. H. Shimizu, A. Kaito, and M. Hatano: *Bull. Chem. Soc. Jpn.* **52**, 2678 (1979).
2. N. Kobayashi, R. Saito, Y. Hino, A. Ueno, and T. Osa: *J. Chem. Soc., Chem. Commun.* 706 (1982).
3. N. Kobayashi, Y. Hino, A. Ueno, and T. Osa: *Bull. Chem. Soc. Jpn.* **56**, 1849 (1983).
4. H. Shimizu, A. Kaito, and M. Hatano: *Bull. Chem. Soc. Jpn.* **54**, 513 (1981).
5. M. Suzuki and Y. Sasaki: *Chem. Pharm. Bull.* **29**, 585 (1981).
6. M. Ata, M. Aoyagi, Y. Kubozono, and Y. Gondo: *Chem. Lett.* **2**, 341 (1989).
7. M. Kajtar, C. Horvath-Toro, E. Kuthi, and J. Szejtli: *Proc. Int. Symp. Cyclodextrins, 1st Meeting*, **1**, 181 (1981).

8. H. Hirai, N. Toshima, S. Hayashi, and Y. Fujii: *Chem. Lett.* **5**, 643 (1983).
9. S. M. Han and N. Purdie: *Anal. Chem.* **56**, 2822 (1984).
10. I. Suzuki, A. Ueno, and T. Osa: *Chem. Lett.* **11**, 2013 (1989).
11. N. Kobayashi and T. Osa: *Carbohydr. Res.* **192**, 147 (1989).
12. K. Kano, H. Matsumoto, Y. Yoshimura, and S. Hashimoto: *J. Am. Chem. Soc.* **110**, 204 (1988).
13. J. Szejtli: *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest (1982).
14. A. Nakajima: *Spectrochim. Acta* **39A**, 913 (1983).
15. K. Kano, I. Takenoshita, and T. Ogawa: *Chem. Lett.* 1035 (1980).
16. R. Breslow, M. F. Czaruiecki, J. Emert, and H. Hamaguchi: *J. Am. Chem. Soc.* **102**, 762 (1980).
17. A. Ueno, K. Takahashi, and T. Osa: *J. Chem. Soc., Chem. Commun.* 921 (1980).
18. L. Blyshak, K. Y. Dodson, G. Patonay, and I. M. Warner: *Anal. Chem.* **61**, 955 (1989).
19. G. Patonay, K. Fowler, G. Nelson, and I. M. Warner: *Anal. Chim. Acta* **207**, 251 (1988).
20. G. Nelson, G. Patonay, and I. M. Warner: *Talanta* **36**, 199 (1989).
21. G. Nelson, G. Patonay, and I. M. Warner: *Anal. Chem.* **60**, 274 (1988).
22. H. Hirai, N. Toshima, and S. Uenoyama: *Bull. Chem. Soc. Jpn.* **58**, 1156 (1985).
23. G. Patonay, M. E. Rollie, and I. M. Warner: *Anal. Chem.* **57**, 569 (1985).
24. *Spectral Atlas of Polycyclic Aromatic Compounds*, Ed.: W. Karcher, R. J. Fordham, J. J. Dubois, P. G. J. M. Glaude and J. A. M. Ligthart, D. Reidel Publishing Company, Boston, 1985.