Carbohydrate Research, 65 (1978) 173–182

A FLUORESCENCE-PROBE STUDY OF THE INTERACTION OF CYCLOHEPTAAMYLOSE WITH ARENES AND AMPHIPHILLIC MOLECULES*

HAYDN E. EDWARDS

North East Wales Institute of Higher Education, Kelsterton College, Connah's Quay, Clwyd CH5 4BR (Great Britain)

AND JOHN K. THOMAS

Chemistry Department and Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556 (U. S. A.)

(Received November 14th, 1977; accepted for publication, November 24th, 1977)

ABSTRACT

The inclusion of the fluorescence probe pyrene within the void of cycloheptaamylose has been demonstrated by the nature of the fluorescence spectra of the arene, the lifetime of the fluorescence, and the rate of reaction of excited states of the arene with quencher molecules. The data suggest two equilibria processes, namely, an initial, fast inclusion of the arene, followed by a slower aggregation of the resulting cycloamylose–pyrene complexes. Addition of surfactant molecules, below the critical micelle concentration (c.m.c.), to the pyrene–cycloamylose solution leads to complexing of the three components of the system and thence to an increase in the hydrophobicity of the arene environment. Above the c.m.c., micelles of the surfactants are formed and the arene is transferred from the cycloamylose to the micelle. The fluorescence measurements establish conditions for varying the hydrophobicity of the arene environment. Preliminary kinetic data show that the rates of quenching of the excited states of the arene by such molecules as I^- and CH_3NO_2 are significantly decreased by inclusion in the dextrin. The data suggest systems that may be used to study molecular details of the reactions of excited states with quencher molecules.

INTRODUCTION

Cycloamyloses (Schardinger dextrins) have attracted attention as compounds that simulate enzyme-like activity[2]. The catalytic action of cycloamyloses is connected with their ability to form inclusion compounds with various molecules[3–6]. The structures of several complexes have been investigated by X-ray diffraction[7] and n.m.r. spectroscopy[8]. It is generally agreed that organic molecules are included in the doughnut-like structure of the cycloamylose. In many ways, this behaviour is

*See ref. 1.

reminiscent of solubilization of hydrophobic molecules by micelles in aqueous solution. An e.s.r. spin-label study[9] points out the similarity for solubilization of a nitroxide free-radical in cycloamyloses and micellar solution.

Certain aggregated systems, such as membranes[10], lipid dispersion[11], and micelles[12], modify the photochemistry of arene molecules solubilized in these systems, and thereby provide data useful in commenting on the nature of the aggregated system. The concept of the present work is to investigate the influence of cycloamyloses on the photochemistry of an included arene molecule, for example, pyrene. The fluorescence spectrum will indicate the polarity of the probe's environment in the cycloamylose, while fluorescence quenching studies with quencher molecules in the aqueous phase will monitor the restriction placed by the cycloamylose on the movement of the probe and quencher.

In particular, cycloamyloses are stable entities in aqueous studies, unlike micelles, which are transitory and are in dynamic equilibrium with the monomer amphiphile from which they are formed. This difference may be reflected in the photochemical data and may prove significant in commenting on the nature of micelle solubilization.

EXPERIMENTAL

Cyclo-hexa- and -hepta-amyloses were obtained from Sigma Chemicals and were used without further purification. Pyrene (Eastman Kodak) was purified by elution from a silica gel column. Water was distilled in a Barnsted still followed by distillation from $KMnO_4$ solution. Sodium lauryl sulfate (NaLS), cetyltrimethylammonium bromide (CTAB), sodium octyl sulphate (NaOS), and sodium cetyl sulphate (NaCS) were purified by recrystallization from ethanol.

Sample preparation. — The solutions were made by injecting a concentrated solution of the probe in ethanol into an aqueous solution of the cycloamylose followed by stirring for 24 h. The fine precipitate of probe resulting from mixing was subsequently included in the cycloamylose. The samples were deoxygenated by bubbling with nitrogen for several minutes.

Apparatus. — Fluorescence spectra were recorded on an Aminco-Bowman spectrofluorimeter, and absorption spectra on a Carey 15 spectrophotometer. Quenching experiments were performed by using a frequency-doubled, Q-switched ruby laser, Korad IQ. The wavelength of the exciting light was 347.1 nm, the duration of the pulse 15 μsec, and the energy of the pulse 250 mJ. The transitory excited states were monitored by fast-emission spectroscopy; the technique has been previously described[13,14].

RESULTS AND DISCUSSION

Fig. 1 shows the uncorrected fluorescence emission spectrum of 10 μM pyrene in water, in a freshly prepared 10mM solution of cycloamylose, and in the latter solution after ageing for 24 h. The solubility[15] of pyrene in water is less than 2μM.

However, relatively stable, aqueous suspensions may be formed at higher concentrations, as shown in Fig. 1.

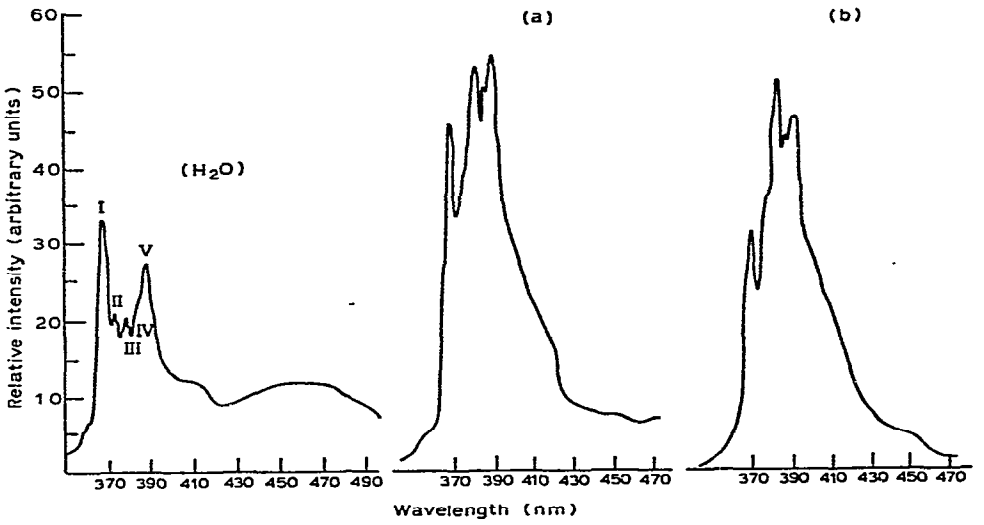

Fig. 1. Uncorrected fluorescence spectra of pyrene (10 μM) in water; and in cycloheptaamylose (10 μM): (*a*) freshly prepared, (*b*) matured. The emission intensities of the spectra are not comparable due to changes in the amplifier settings.

The fluorescence spectrum contains a region from ~370 to 410 nm which is the monomer fluorescence of pyrene[16]. A subsequent, broad spectral region (~410 to 500 nm) is that of the excited dimer or excimer of pyrene. At similar concentrations of pyrene in alkanes or alcohols, only the monomer fluorescence (370–410 nm) is observed; the excimer fluorescence observed in water at 10 μM is associated with the formation of clusters of pyrene, *i.e.*, the solution is not truly a mono-molecularly dispersed pyrene solution. The excimer fluorescence disappears on addition of cycloamylose, and the structural details of the monomer fluorescence are altered. It has been shown previously that the structural details and the intensity of the pyrene monomer fluorescence vary with the polarity of the solvent[17,18].

If the peaks are numbered I to V as shown in Fig. 1, the ratio of peaks III and I is 0.63 in water and 1.65 in hydrophobic solvents. The III/I ratio has been used to investigate the polarity of the pyrene environment in micelles and membranes[18], and is used here to investigate the environment of pyrene in cycloamyloses. Fig. 1 shows that pyrene in water shows considerable excimer emission and a III/I ratio of 0.63. Addition of 10mM cycloamylose almost eliminates the excimer emission and also changes the III/I ratio to 1.17. Ageing this solution gives rise to a further small change in the fluorescence spectrum; Fig. 1b shows the emission from a matured solution after 24 h, where the III/I ratio is 1.63. Table I gives the change in emission properties with time.

TABLE I

THE CHANGES IN THE III/I AND E/M RATIOS OF PYRENE (10 μM) IN CYCLOHEPTAAMYLOSE (10mM) WITH TIME

Duration of stirring (h)	0	3	7	11	24	48[a]
III/I	1.03	1.20	1.28	1.42	1.49	1.65
E/M	0.137	0.078	0.067	0.05	0.05	0.03

[a]Stirring for 24 h, and standing for a further 24 h.

The data indicate that pyrene suspended in water is slowly solubilized by the dextrin, and that the environment becomes quite hydrophobic. The degree of solubilization of pyrene by cycloamylose may be conveniently followed by the III/I ratio or the excimer/monomer E/M ratio (ratio of emission at 470/392 nm). At low concentrations of pyrene, the E/M ratio is not high and corrections are not required. On increasing the pyrene concentration the excimer spectra and monomer spectra overlap, and complications arise. In this work, the concentrations of pyrene were selected so that overlapping did not occur.

Fig. 2 shows the effect of cycloamylose concentration on solubilizing different concentrations of pyrene, as measured by the E/M ratio. For pyrene concentrations $\geqslant 10$ μM, significant solubilization is only achieved above 3mM cycloamylose. Similar data are shown in Fig. 3, using the III/I ratio to estimate the degree of solubilization. Below 3mM dextrin, the III/I ratio decreases and approaches that observed in water. The data with aged and freshly prepared solutions are similar in shape, showing a change in III/I ratio at 3mM cycloamylose.

The effect of increasing the concentration of pyrene, at constant cycloamylose concentration, on the III/I ratio and pyrene fluorescence lifetime (τ) is shown in Fig. 4. An increased τ is also associated with decreasing polarity of the pyrene environment. Both the III/I ratio and τ increase with increasing concentration of pyrene, particularly at the lower concentrations of pyrene in the 100 μM region. At mM pyrene, the III/I ratio is 2.0 and approaches that observed in the gas phase or in perfluoro solvents, where the interaction of solute and solvent is very small. All data are best interpreted by an interaction of pyrene and cycloamylose.

Pyrene suspended in water is included by cycloamyloses, most probably in the centre of the doughnut-like cycloamylose structure. It is suggested that one molecule of pyrene is initially associated with one molecule of cycloamylose. Such a structure still leaves opportunity for the pyrene to interact with a limited amount of water at the open end of the cycloamylose structure. However, the environment is much less polar than water, giving rise to an increase in τ and in the III/I ratio. The slow solubilization that has been observed previously in similar systems[5] may be due to the difficulty in inserting the pyrene into the small vacancy in the cycloamylose centre.

The ageing effect could also be due to the gradual penetration of pyrene into the cycloamylose, which will lead to a more hydrophobic environment.

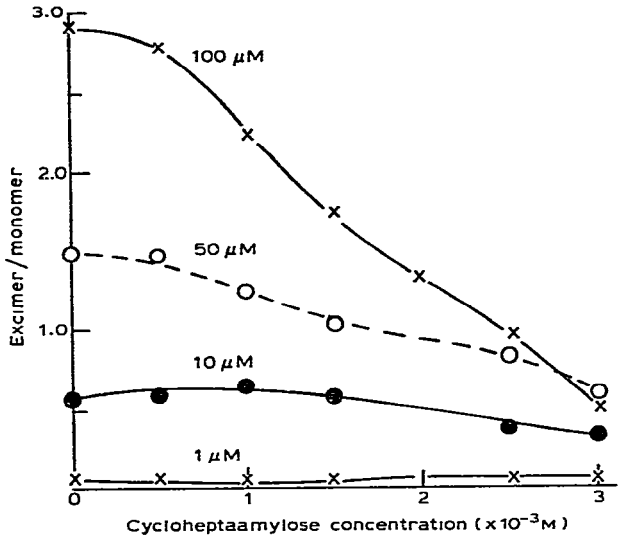

Fig. 2. Changes in the excimer/monomer ratio of pyrene at various concentrations with increasing concentrations of cycloheptaamylose.

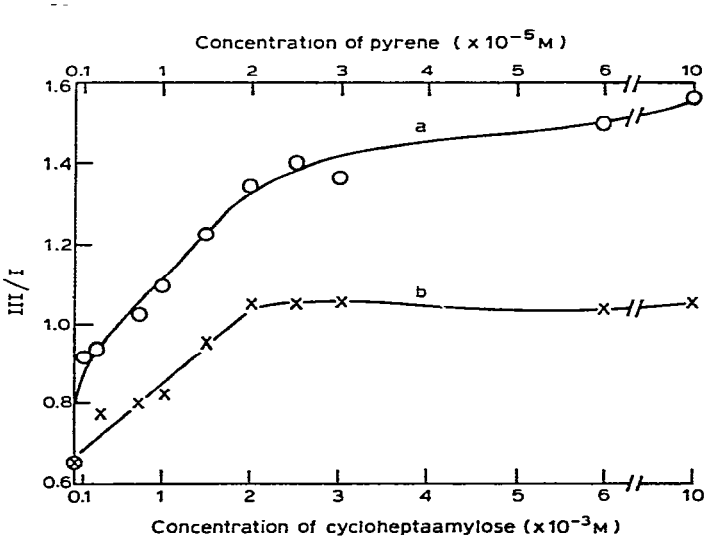

Fig. 3. Changes in the III/I ratio of pyrene (100 μM)/cycloheptaamylose (10mM) following dilution with water: (a) matured and (b) freshly prepared.

The equilibrium may be represented as

$$\text{Pyrene} + \text{Cycloamylose} \rightleftharpoons \text{Complex } \mathbf{1}. \qquad (1)$$

Process *1* is almost complete at ~3mM cycloamylose. Increasing the pyrene concentration increases the concentration of complex **1**, and a second association takes place to form complex **2**.

$$2\ \text{Complex } \mathbf{1} \rightleftharpoons \text{Complex } \mathbf{2} \qquad (2)$$

Complex **2** is composed of several cycloamylose and pyrene molecules. As the III/I ratio and τ increase on forming complex **2**, the environment of the pyrene molecules must become more hydrophobic, and contact with water *via* the open ends of the cycloamylose is minimised. Fig. 2 shows how the yield of pyrene excimers increases with pyrene concentration at several concentrations of cycloamylose, and the decrease in τ above 200 μM pyrene may be due to excimer formation as the pyrene molecules are brought into further contact by formation of complex **2**. Processes *1* and *2* predict the following relationship for concentrations of all species:

$$[\text{Complex } \mathbf{2}]/[\text{Complex } \mathbf{1}] = K_2 K_1\,[\text{Pyrene}]\,[\text{Cycloamylose}],$$

where K_2 and K_1 are equilibrium constants for processes *2* and *1*, respectively. It is stated that the pyrene environment in complex **2** is more hydrophobic than that in

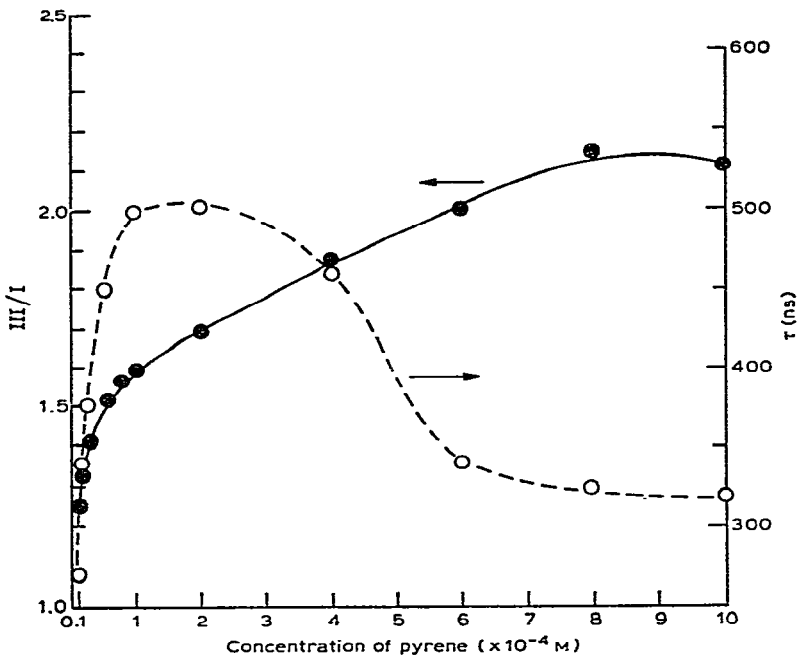

Fig. 4. The effects of increasing the pyrene concentration in aqueous solutions of cycloheptaamylose (10mM) on the τ and III/I ratio of pyrene.

complex **1**. The increase in III/I ratio in Fig. 4 monitors the increase in complex **2** at the expense of **1**. An increase in [pyrene] or [cycloamylose] leads to an increase in this ratio, as required by the above relationship.

In Fig. 4, the lowest [pyrene] of 10 μM is taken as pure complex **1**, III/I = 1.25, and a III/I ratio of 2.15 is extrapolated for infinite pyrene, *i.e.*, pure complex **2**. The

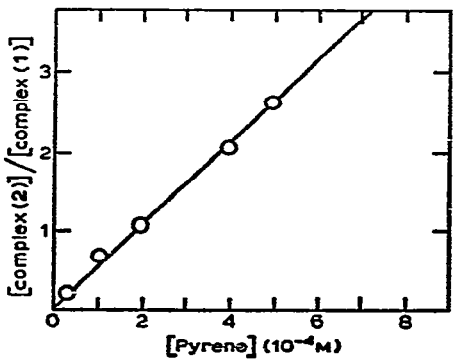

Fig. 5. Plot of the ratio of [complex 2]/[complex 1] against pyrene concentration.

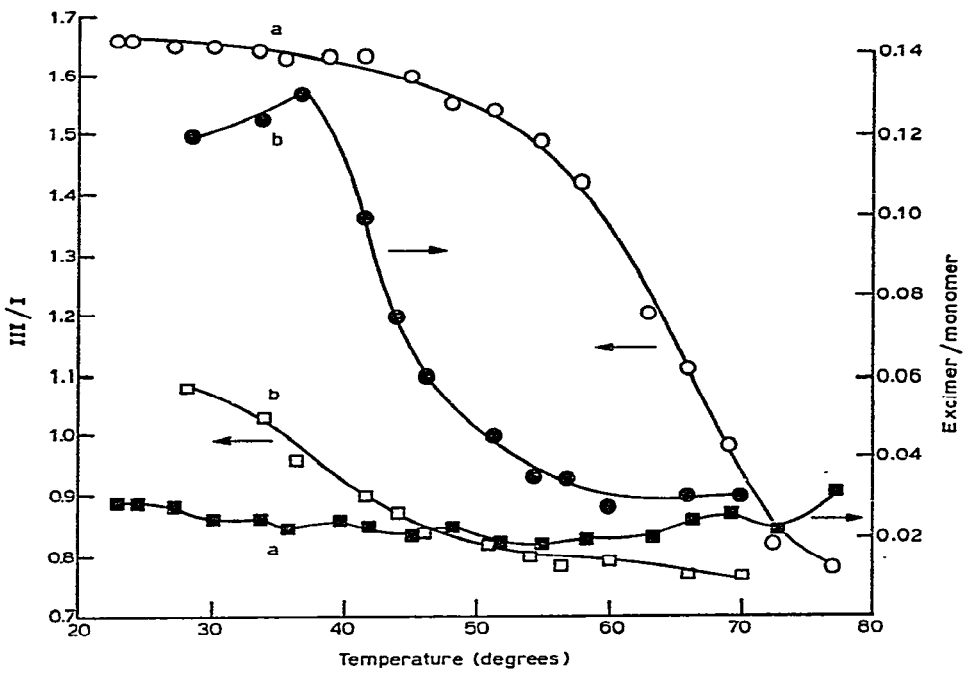

Fig. 6. Effect of increasing temperature on the excimer/monomer ratio of pyrene (10 μM) in aqueous solutions of cycloheptaamylose (10mM): (a) mature and (b) freshly prepared.

III/I ratio change is then indicative of the change in [complex 2]/[complex 1]. This is plotted in Fig. 5 versus [pyrene], and gives a linear plot. The slope of the line gives the product $K_2K_1 = 5.2 \times 10^5$ M^{-2}. The effect of temperature on the pyrene/cycloamylose system is shown in Fig. 6 for matured and freshly prepared solutions. For both solutions, increasing temperature leads to a decrease in the III/I ratio. In these experiments, the E/M ratio does not increase as the pyrene moves into the aqueous phase from complex 1. Any excimers formed by this process are dissociated by the increased temperature. This can be demonstrated by heating a solution of 10 μM pyrene in water. The excimer band gradually decreases with a comcomitant rise in the monomer fluorescence. The excimer does not immediately reappear on cooling the solution, and an unstable suspension of pyrene is formed. Several hours are needed to re-establish the equilibrium normally observed at room temperature.

It has been reported that many amphiphatic molecules, *e.g.*, surfactants, bind efficiently to cycloamyloses[6]. Fig. 7 shows the effect of the sodium lauryl sulphate (NaLS) on the fluorescence of pyrene/cycloamylose complexes. The III/I fluorescence ratio and the percentage change of the fluorescence in the presence and absence of oxygen ($I_q/I_o \times 100$) of the mixture compared to that in the absence of surfactant are plotted versus surfactant concentration.

All experiments were carried out in O_2-saturated solution where the quenching of excited pyrene is much faster in micelles than in the cycloamylose complex[12]. Hence,

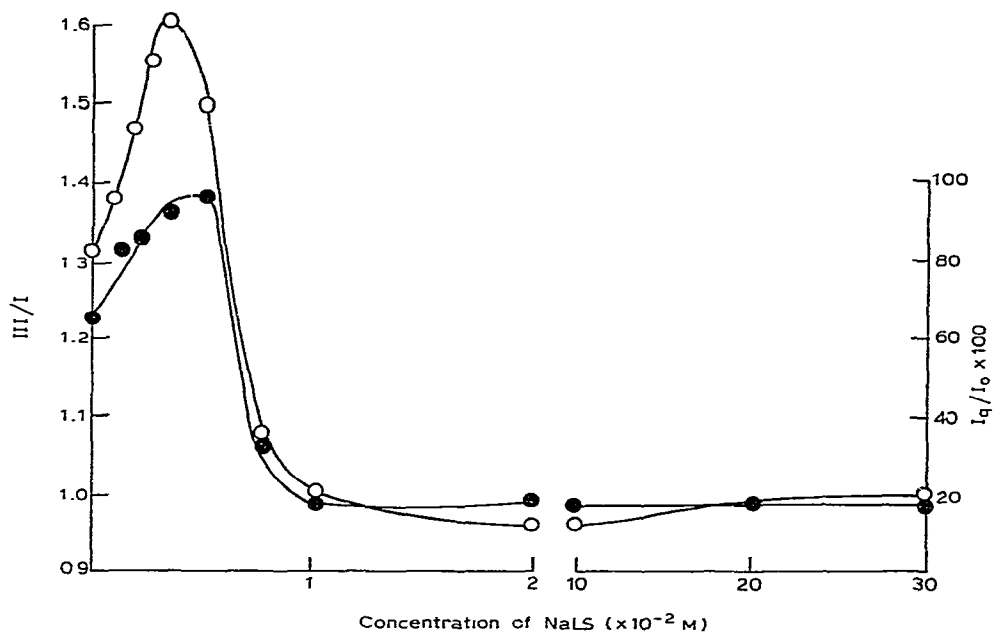

Fig. 7. The effect of increasing concentrations of NaLS on the changes in the III/I ratio of pyrene and the % decrease in the pyrene monomer intensity at 392 nm after saturation of sample with oxygen: cycloheptaamylose (5mM), pyrene (5 μM). Ex. λ—310 nm.

a change in the pyrene location from the cycloamylose to the micelle leads to a decrease in the fluorescence yield. Both the III/I ratio and fluorescence intensity initially increase to a maximum on addition of surfactant, followed by a gradual decrease to values expected for micellar solution. The data are similar for other surfactants used, with the maxima occurring in regions close to the c.m.c. of the surfactant: 8mM for NaCS, 7.5mM for NaLS, 0.15M for NaOS, and 3mM for CTAB. The initial increase in III/I ratio and fluorescence intensity are interpreted as a complexing of the surfactant with complex **1** to provide a further shielding of the pyrene from the water environment and the oxygen. At the onset of micelle formation, the pyrene is removed from the cycloamylose complex and resolubilized in the micelle. The fluorescence intensity and the III/I ratio revert to values observed for micelles. The data show that solubilization of pyrene in micelles is more favourable than in cycloamylose complexes.

Solubilization of pyrene in the cycloamylose complex protects the excited state from quenching molecules solubilized in the aqueous phase. Table II lists the rate constants for quenching excited pyrene produced by laser flash photolysis in 10mM and mM cycloamylose by O_2, I^-, Tl^+, and CH_3NO_2. The quenching rate-constants decrease with increasing concentration of cycloamylose from 1mM to 10mM, and are indicative of a further screening of the pyrene by cycloamylose due to more than one cycloamylose molecule complexing around the pyrene. These data are analogous to those in Fig. 3, where increasing concentration of cycloamylose leads to an increase in the III/I ratio, *i.e.*, an increase in the hydrophobicity of the pyrene environment, and a shielding of the chromophore from the aqueous phase containing the quencher.

TABLE II

QUENCHING OF PYRENE (10^{-5} M) IN CYCLOAMYLOSE ($\times 10^9$ MOL^{-1}.SEC^{-1})

		Cycloheptaamylose		*Methanol*
		(*10mM*)	(*mM*)	
Pyrene	O_2	2.35	5.4	20
	I^-	0.02	0.61	4.5
	NO_2	0.78	7.9	10
	Tl^+	0.45	11	20

CONCLUSION

The foregoing data conclusively show that pyrene is included by cycloheptaamylose, most probably in the core of this doughnut-shaped molecule. Formation of the complex is not a simple process, as the pyrene environment becomes increasingly hydrophobic as both pyrene and cycloamylose concentrations are increased. The data indicate a continued aggregation of the 1:1 pyrene/cycloamylose complex in the 1:1 complex. The ends of the long axis of pyrene protrude into the aqueous phase.

Continued aggregation or further complexation by surfactant molecules removes the pyrene completely from the aqueous environment. Under such conditions, the environment of pyrene resembles that in fluorocarbons or in the gas phase where little solvent–solute interaction occurs.

ACKNOWLEDGMENTS

One of the authors (H.E.E.) acknowledges the support of E.R.D.A., and the use of the facilities in the Radiation Laboratory, University of Notre Dame.

REFERENCES

1 The research described herein was supported by the Division of Physical Research of the U.S. Energy Research and Development Administration. This is Radiation Laboratory Document No. NDRL-1754.
2 D. W. GRIFFITHS AND M. L. BENDER, *Adv. Catal.*, 23 (1973) 209–261.
3 D. FRENCH, *Adv. Carbohydr. Chem.*, 12 (1957) 189–260.
4 T. A. THOMAS AND L. STEWARD, in R. L. WHISTLER (Ed.), *Starch: Chemistry and Technology*, Vol. 1, Academic Press, New York, 1965, p. 209.
5 H. SCHLENK AND D. M. SAND, *J. Am. Chem. Soc.*, 83 (1961) 2312–2320.
6 T. OKUBO, H. KITANO, AND N. ISE, *J. Phys. Chem.*, 80 (1976) 2661–2664.
7 A. HYBL, R. E. RUNDLE, AND D. E. WILLIAMS, *J. Am. Chem. Soc.*, 87 (1965) 2779–2788.
8 P. V. DEMARO AND A. L. THAKKER, *Chem. Commun.*, (1970) 2–4.
9 N. ATHERTON AND S. J. STARCH, *J. Chem. Soc., Faraday Trans. 1*, 71 (1975) 357–360.
10 S. CHENG, J. K. THOMAS, AND C. F. KULPA, *Biochemistry*, 13 (1974) 1135–1139.
11 S. CHENG AND J. K. THOMAS, *Radiat. Res.*, 60 (1974) 268–279; D. W. BARBER, D. A. N. MORRIS, AND J. K. THOMAS, *Chem. Phys. Lett.*, 37 (1976) 481–484.
12 S. C. WALLACE AND J. K. THOMAS, *Radiat. Res.*, 54 (1973) 49–62; M. GRATZEL AND J. K. THOMAS, *J. Am. Chem. Soc.*, 95 (1972) 6885–6889.
13 R. MCNEIL, J. T. RICHARDS, AND J. K. THOMAS, *J. Phys. Chem.*, 74 (1970) 2290–2294.
14 J. K. THOMAS, *Rec. Chem. Progr.*, 32 (1971) 145–163.
15 W. W. DAVIS, M. E. KRAHL, AND B. H. A. CLOWES, *J. Am. Chem. Soc.*, 64 (1942) 108–110.
16 I. S. BERLMAN, *Handbook of Fluorescence of Aromatic Molecules*, Academic Press, New York, 2nd edition, 1976, p. 383.
17 A. NAKAJIMA, *J. Mol. Spectrosc.*, 61 (1976) 467–469.
18 K. KALYANASUNDARAM AND J. K. THOMAS, *J. Am. Chem. Soc.*, 99 (1977) 2039–2044.