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Absorption spectra of pyrene in bovine serum albumin and caffeine aqueous solutions

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Abstract

The interactions between pyrene and bovine serum albumin (BSA) and pyrene and caffeine were studied using absorption spectroscopy in aqueous solutions. In the pyrene–BSA system, an equilibrium between free and adsorbed pyrene was observed in the solution. The absorption spectrum of pyrene adsorbed by BSA was obtained. The equilibrium constant was determined to be 1.1×10^6 dm^3 mol^{-1}. In the presence of caffeine, the absorption spectrum was a superposition of three species; free pyrene and 1:1 and 1:2 pyrene–caffeine complexes. The equilibrium constants were determined to be 820 and 260 dm^3 mol^{-1} for the formation reactions of 1:1 and 1:2 pyrene–caffeine complexes respectively.

Keywords: Absorption spectra; Pyrene; Bovine serum albumin; Caffeine; Complex

1. Introduction

Aromatic hydrocarbons are strongly hydrophobic compounds which are poorly soluble in water [1,2]. However, if aromatic molecules form complexes with hydrophilic compounds, the apparent solubilities can be increased significantly. Biopolymers usually have hydrophobic and hydrophilic parts within the molecule. Complex formation may be expected between many aromatic molecules and biomolecules. Various investigations have been reported on complex formation between simple aromatic molecules and biorelated molecules, such as cyclodextrin [3], albumin [4] and purine [5,6].

The apparent solubility of pyrene in water increases when bovine serum albumin (BSA) is added. The absorption spectra, fluorescence spectra and fluorescence lifetimes of pyrene in BSA aqueous solutions have been reported [4]. With increasing concentration of BSA, the absorption bands shift to the red. The fluorescence spectra and lifetimes show that the polarity of the binding site changes from polar to nonpolar in the presence of BSA.

Quantitative studies on the solvent effects of some aromatic molecules with purine have been performed using fluorescence measurements. The formation of 1:1 complexes is common. For benzopyrene and pyrene, solubilization by caffeine can be accounted for by the formation of both 1:1 and 1:2 complexes.

The structures of the complexes between aromatic molecules and purine have been studied by nuclear magnetic resonance (NMR) spectroscopy [7–9]. The structures of the complexes between 1,3,7,9-tetramethyluric acid (TMU) and aromatic molecules were shown to be near face to face, except for the TMU–benzene complex which showed a perpendicular arrangement [7]. The complexes may be predominantly formed by dipole–induced dipole interactions and the existence of charge transfer complexes was excluded. However, opposing views exist [10–13].

2. Experimental details

Pyrene was purchased from Aldrich. BSA was obtained from Sigma as the fatty-acid-free and charcoal-filtered grade. Caffeine was supplied by Kanto Chemicals as the anhydrous grade. They were used as received.

Pyrene aqueous solutions were prepared by mixing pyrene ethanol solution with various amounts of water. The sample solutions were prepared containing an amount of ethanol less than 0.1% of the total volume; they were considered to be aqueous solutions [4].

The absorption spectra were measured by a spectrophotometer with a 10 cm cylindrical absorption cell. The sample

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temperature was maintained at 25 °C. The fluorescence spectra were measured with a spectrofluorometer. Time-resolved fluorescence spectra were measured by the single-photon counting method. All sample solutions were degassed before measurement.

3. Results and discussion

3.1. Pyrene in BSA aqueous solution

Although pyrene is a strongly hydrophobic compound, it dissolves in water with a concentration up to about 10^{-6} mol dm^{-3} at room temperature [1,2]. In Fig. 1, the full line is the absorption spectrum of pyrene aqueous solution. The absorption bands appearing at around 334 nm and 320 nm are the well-known vibrational structures of pyrene in polar solution [14].

In the presence of BSA, the absorption bands of pyrene are slightly red shifted as shown in Fig. 1 for three different BSA concentrations. The clear isosbestic point at 336 nm indicates that an equilibrium between two different forms of pyrene is established: the spectra are superpositions of the absorption bands of adsorbed and unadsorbed pyrene.

Analysis of the absorption spectra of pyrene in the absence of BSA and in the presence of a large excess of BSA was performed and the concentrations of the components were determined. The results show that, with increasing concentration of BSA, the fraction of pure pyrene absorption decreases and that of adsorbed pyrene on BSA increases. For BSA concentrations above 1.5×10^{-6} mol dm^{-3}, nearly 100% of pyrene molecules are adsorbed by BSA. A fairly large equilibrium constant, 1.1×10^{6} dm^{3} mol^{-1}, was estimated from the data.

3.2. Pyrene in caffeine aqueous solution

As shown in Fig. 2, the absorption spectrum of pyrene in the presence of caffeine shows a similar red shift to the pyrene–BSA system. The strong absorption appearing in the shorter wavelength region is due to caffeine.

The results shown in Fig. 2 indicate that pyrene and caffeine molecules also form complexes. However, unlike the pyrene–BSA system, at least two kinds of complex must be formed between pyrene and caffeine because of the absence of an isosbestic point.

For further discussion, it was assumed that the pyrene–caffeine system is composed of three species, py, py–ca and ca–py–ca, where py stands for free pyrene, py–ca is a 1:1 complex and ca–py–ca is a 1:2 complex formed between pyrene and caffeine molecules.

An equilibrium should be established between these species

$$\text{py} + \text{ca} = \text{py–ca} \qquad K_1 \qquad (1)$$

$$\text{py–ca} + \text{ca} = \text{ca–py–ca} \qquad K_2 \qquad (2)$$

where K_1 and K_2 are the equilibrium constants. The observed absorption spectrum is then given simply by the sum of four absorption spectra: those of py, py–ca, ca–py–ca and ca, taking each concentration into account.

The best-fit absorption spectra for various caffeine concentrations can be reproduced by substituting the appropriate absorption spectra and the concentrations determined by Eqs. (1) and (2). Since the absorption spectra of free pyrene and caffeine are known, the problem is reduced to the determination of the best-fit values of K_1, K_2 and the absorption spectra of py–ca and ca–py–ca complexes to the observed absorption spectrum. Using 12 sets of absorption spectra for different caffeine concentrations, the best-fit parameters K_1 and K_2 and the absorption spectra of py–ca and ca–py–ca complexes (each absorption spectrum expressed by a set of 400 points) were determined numerically by least-squares calculation.

The absorption spectra of the pyrene–caffeine complexes are shown in Fig. 3. The deconvoluted spectra resemble the spectrum of free pyrene with slight red shifts (3.4 nm and 6.7 nm respectively). The best-fit K_1 and K_2 values are 820 and 260 dm^{3} mol^{-1} respectively.

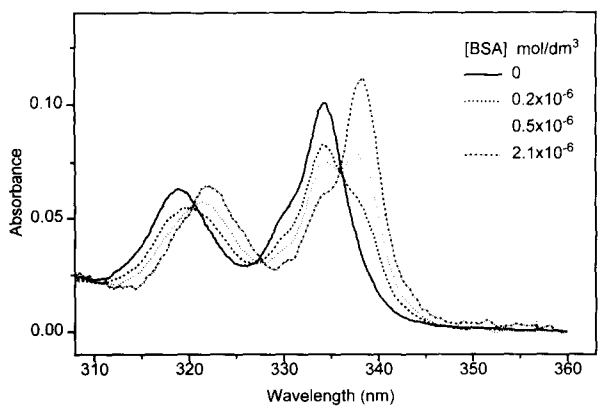

Fig. 1. Absorption spectra of pyrene (2×10^{-7} mol dm^{-3}) at various concentrations of BSA in aqueous solution.

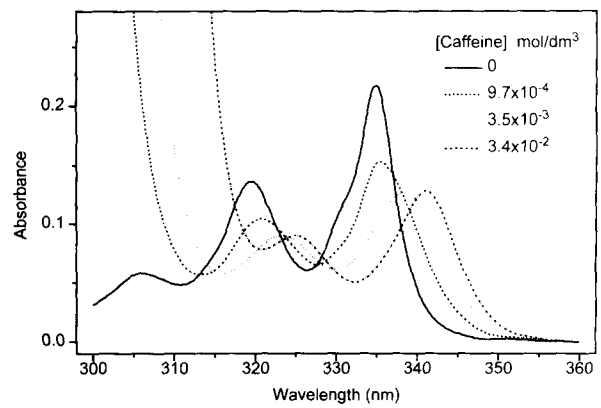

Fig. 2. Absorption spectra of pyrene (4×10^{-7} mol dm^{-3}) at various concentrations of caffeine in aqueous solution.

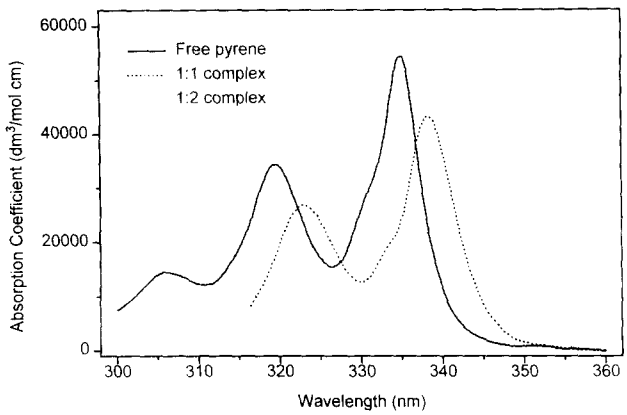

Fig. 3. Absorption spectra of free pyrene, 1:1 and 1:2 pyrene–caffeine complexes.

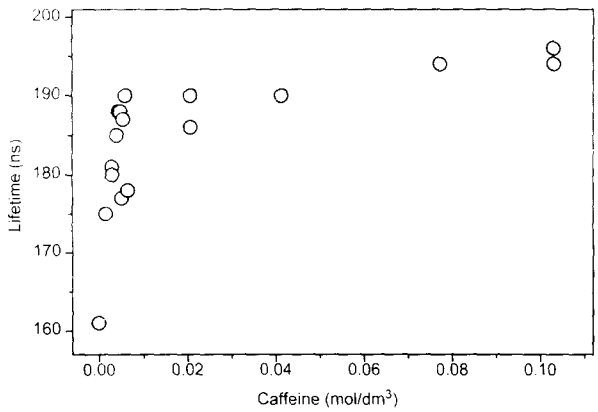

Fig. 4. Fluorescence lifetime of pyrene (2×10^{-7} mol dm^{-3}) at various concentrations of caffeine in aqueous solution.

The fluorescence spectra and decay curves of pyrene aqueous solutions were measured at various caffeine concentrations. The ratio of the first and third vibronic bands of the fluorescence spectrum increases as the caffeine concentration increases. However, these changes are not significant compared with the spectral changes observed in the pyrene–BSA system [4]. These results indicate that the pyrene–caffeine complexes yield only slightly less polar media for pyrene, while the character of pyrene in BSA is nearly that in a nonpolar medium.

The fluorescence lifetimes shown in Fig. 4 also support this conclusion. As the caffeine concentration increases to 0.01 mol dm^{-3}, the lifetime rapidly increases from 160 to 190 ns. At this caffeine concentration, the concentration of free pyrene decreases and that of py–ca increases, while the ca–py–ca concentration is still low. For higher caffeine concentrations than 0.01 mol dm^{-3}, the lifetime increases very gradually to about 195 ns at 0.1 mol dm^{-3}. Thus the fluorescence lifetimes of py–ca and ca–py–ca complexes are about 190 ns and 195 ns respectively. The lifetime of 360 ns reported for the pyrene–BSA system is close to that in nonpolar solutions [16].

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