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ORIGINAL ARTICLE

Host properties of cucurbit [7] uril: fluorescence enhancement of acridine orange

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Abstract The inclusion behavior of cucurbit [7] uril with acridine orange was investigated by fluorescence spectrometry in acetate buffer solution. It was found that the fluorescence intensity of acridine orange regularly increased upon the addition of cucurbit [7] uril accompanying with blue-shift of the position of the emission maximum. The results indicate the formation of the complex at a 1:1 complex stoichiometry and the association constant was calculated by applying a deduced equation. From the temperature dependence of the equilibrium constants, ΔH and ΔS values were obtained, indicating an enthalpic driving force for complexation. Meanwhile, the possible interaction mechanism was also discussed.

Keywords Cucurbit [7] uril · Acridine orange · Fluorescence · Inclusion interaction · Thermodynamics

Introduction

Cucurbiturils are macrocyclic container molecule consisting of N (n = 5–10) glycoluril monomers joined by pairs of methylene bridges [1]. They have a highly symmertrical structure which is similar to cyclodextrins [2] and calixarenes [3]. However, comparing with the comprehensive study of the other host molecules, cucurbiturils have been gradually established as versatile host molecules for the inclusion of smaller guest molecules [4–9]. In several cucurbituril homologues, cucurbit [7] uril (CB7, Chart 1) has attracted more attraction than others. In the last few years, the host–guest inclusion complexes of cucurbit [7] uril including cations [10, 11], neutral species [12–14] in solution, have been investigated extensively. However, relatively few literatures have been reported about the effect of cucurbit [7] uril on the fluorescence behavior of fluorescent substances and particular common fluorescent dye [15, 16].

Acridine Orange (AO, Chart 1), one of the mostly known acridine fluorescent family members which with well fluorescent properties, provides a straightforward technique to identify plasmodium-infected reticulocytes from infected erythrocytes, uninfected reticulocytes, and white blood cells [17–19].

In this paper, we report the characteristics of host–guest complexation between cucurbit[7]uril (CB7) and the fluorescent dye Acridine Orange (AO) in acetate (HAc–NaAc) buffer solution (0.05 M, pH = 4.70). When CB7 were added to the aqueous solution of AO, an obvious increase in fluorescence intensity and a blue shift were observed. The results indicate AO form a 1:1 stoichiometry complex with CB7. The thermodynamics parameters associated with the inclusion processes of AO in the cavity of the CB7 were also presented, which suggests an enthalpic driving force for complexation related to dipole–dipole interactions, desolvation of the cation and the removal of high-energy water molecules from the CB7 cavity. This work may extend the application range of cucurbit [7] uril in biochemistry.

Experimental

Materials

All reagents used were of analytical-reagent grade. Doubly distilled water was used thoroughly. Cucurbit [7] uril was

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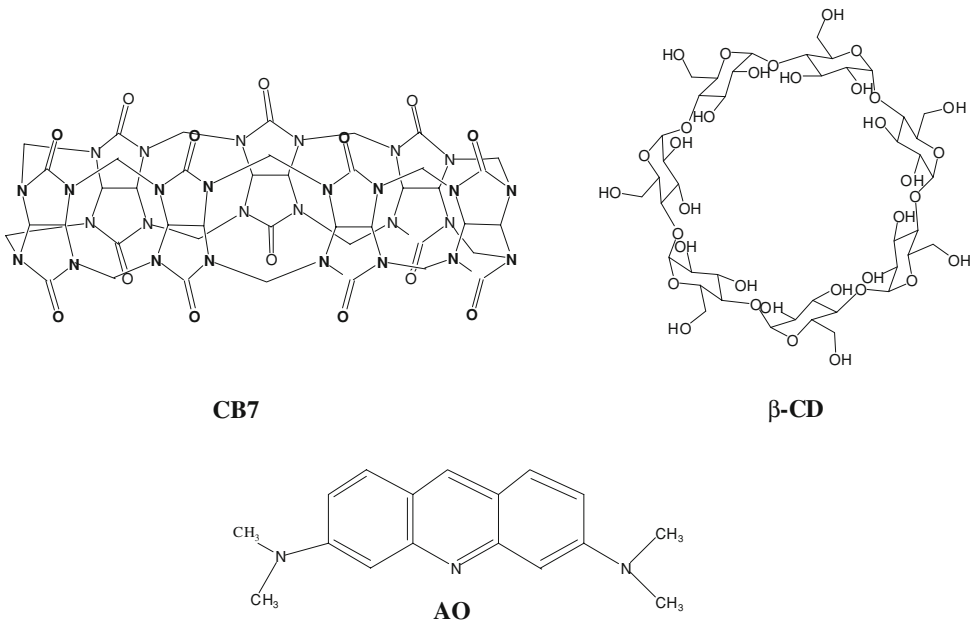
Chart 1 Chemical structures of cucurbit [7] uril, β-cyclodextrin and acridine orange



synthesized according to the reported modified procedure [20–22] and identified by IR, ^{1}H NMR and element analysis. Stock solutions of CB7 were prepared as 1×10^{-3} mol L^{-1}. AO was obtained from Shanghai Chemical Reagent Co., China, and 1×10^{-3} mol L^{-1} stock solution was prepared in water. All the working solutions were prepared by diluting the stock solutions to the concentration required. NaAc–HAc buffer solution (pH = 4.70) was prepared by mixing 0.05 mol L^{-1} sodium acetate solutions with acetic acid.

Apparatus

Fluorescence spectra and relative fluorescence intensities were measured on a model F-4500 fluorescence spectrophotometer (Hitachi, Japan) using a conventional 1×1 cm quartz cell. Excitation and emission bandwidths were set to 5 and 10 nm, respectively. All measurements were carried out at desired temperature adjusted by use of a thermostatic cell holder. Absorption spectra were measured on a U-3010 UV-Visible spectrophotometer (Hitachi, Japan). A model pHS-3C (Dazhong Analytical Instruments Factory, Shanghai, China) pH meter was used for accurate adjustment of pH.

Procedure

A 0.5 mL of 1.00×10^{-4} mol L^{-1} stock AO solution, 1 mL of NaAc–HAc buffer solution (pH = 4.70) and an appropriate amount of 1.00×10^{-4} mol L^{-1} CB7 were transferred into a 10 mL volumetric flask in turn. The mixed solution was diluted to final volume with doubly distilled water and stirred thoroughly. Fluorescence intensities (or absorption spectra) were determined after 15 min at room temperature.

Results and discussion

Fluorescence spectra and absorption spectra characteristics

Experiments expose the AO has strong fluorescence in NaAc–HAc buffer solution (pH = 4.70), as shown in Fig. 1, the fluorescence excitation and emission of AO are 492 and 539 nm, respectively. When an appropriate amount of 1.00×10^{-4} mol L^{-1} CB7 was added into it, the fluorescence spectrum of AO was found to increase significantly in intensity, meanwhile a obviously position

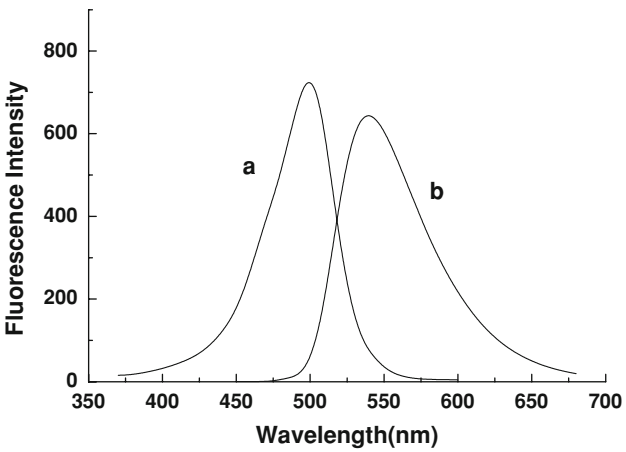


Fig. 1 Fluorescence excitation (a) and emission (b) spectra of AO (5.00×10^{-6} mol L^{-1}) in acetate buffer solution

of the emission maximum blue shift was observed (539 → 521 nm) (see Fig. 2).

In aqueous solution, AO exists a monomer–dimer equilibrium. Figure 3 shows the absorption spectra of AO monomer (488 nm) and dimer (470 nm). In the presence of CB7, the original absorption maximum of AO at 488 nm has a large increase absorption and a slightly shift to shorter wavelength by 5 nm. Meanwhile, the AO dimer peak was gradually invisible with increasing concentration of CB7. This indicated that CB7 enhanced the absorbance of the AO monomer by the formation of the complex and suppressed the dimer formation by inclusion of the AO monomer in the CB7 cavity [23].

According to these results and further study, we suppose that the fluorescence and absorption spectra changes described above are probably induced by the formation of inclusion complexes.

Stoichiometry and inclusion constant

In the enhancement of guest (G) fluorescence upon addition of a non-fluorescent host (H) as a result of the formation of 1:1 host:guest ($[H \cdot G]$) inclusion complex, the measured enhancement depends on the added host concentration according to the equation [24, 25]

$$F/F_0 = 1 + (F_\infty/F_0 - 1)\frac{[H]K}{(1 + [H]K)} \quad (1)$$

where F_∞/F_0 is the fluorescence enhancement when 100% of the guest has been included. Here F_0 is the fluorescence intensity of AO in the absence of CB7, while F is the observed fluorescence intensity at each host molecules concentration tested. And K is the equilibrium binding constant for the complexation:

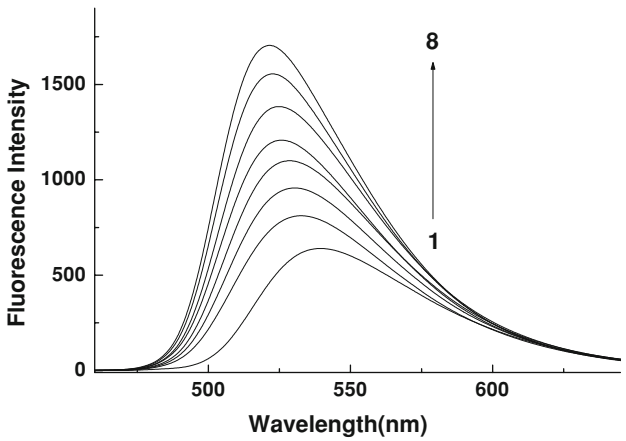


Fig. 2 Fluorescence spectra of AO with different concentration of CB7: from (1) to (8): (1) 0.00, (2) 1.00, (3) 1.50, (4) 2.00, (5) 2.50, (6) 3.00, (7) 4.00, (8) 5.00 × 10^{-6} mol L^{-1}. C_{AO} = 5.00 × 10^{-6} mol L^{-1}

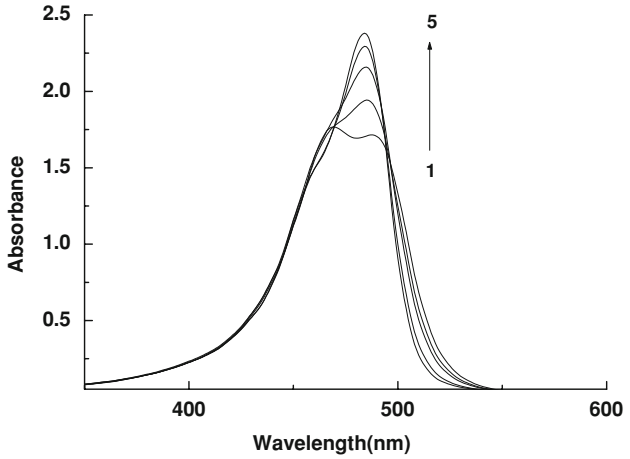


Fig. 3 Absorption spectra of AO with different concentrations of CB7: from (1) to (5): (1) 0.00, (2) 1.50, (3) 2.50, (4) 4.00, (5) 5.00, (6) 6.00 × 10^{-5} mol L^{-1}, C_{AO} = 5.00 × 10^{-5} mol L^{-1}

$$H + G \rightleftharpoons H \cdot G \quad (2)$$

$$K = \frac{[H \cdot G]}{[H][G]} \quad (3)$$

The Fig. 4 shows the enhancement of AO fluorescence as a function of added CB7. The inset shows the linear double reciprocal plot of $1/(F/F_0 - 1)$ vs. $1/[H]$ (r = 0.9991), which confirms the 1:1 stoichiometry of the complex (the applicability of Eq. 1, which will be no linear if higher-order complexes form). The solid line in Fig. 4 shows the excellent fit to Eq. 1, in this case giving K = 1.70 × 10^4 L mol^{-1} and F_∞/F_0 = 3.84. Three such trials were performed, yielding

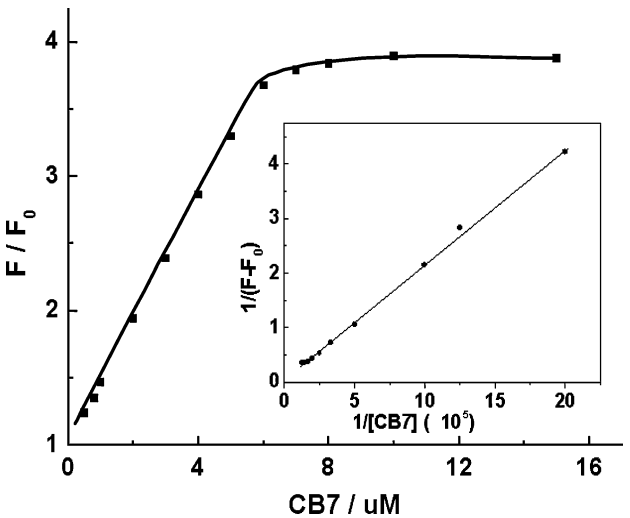


Fig. 4 A fluorescence enhancement, F/F_0 of AO as a function of CB7 concentration. The solid line shows the best fit of the data to Eq. 1: K = (1.70 ± 0.30) × 10^4 L mol^{-1}, F_∞/F_0 = 3.80 ± 0.20. The inset is a plot of $1/(F/F_0 - 1)$ vs. $1/[CB7]$ for CB7-AO complex, which shows the linear double reciprocal plot indicating 1:1 complexation

the average values of $K = (1.70 \pm 0.30) \times 10^4$ L mol^{-1} and $F_\infty/F_0 = 3.80 \pm 0.20$.

The 1:1 inclusion complexes stoichiometry was also established by the continuous variation method (Job's plot) using fluorescence spectroscopy. The solutions of AO and CB7 were mixed in different mole ratio keeping the sum of the AO and host concentrations constant and the maximal relative fluorescence intensity occurred at [AO]/([CB7]) + [AO]) = 0.5. A resulting plot for AO-CB7 complex is shown in Fig. 5.

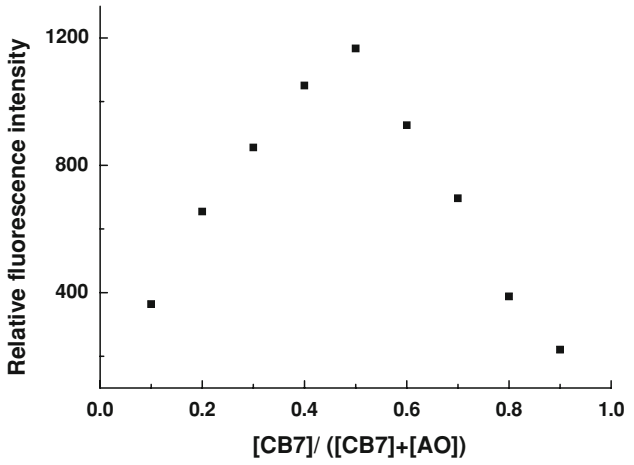


Fig. 5 Job's plot of CB7-AO complex system. Symmetric plot with maximum at 0.5 mole fraction indicate the 1:1 inclusion complex formation in the present system

Thermodynamic parameters of the CB7 inclusion complexes

Thermodynamics Enthalpy, Entropy, and Gibbs free energy values were obtained from the inclusion reaction measuring the fluorescence spectra of AO in presence of CB7 at different temperatures (Fig. 6). The binding constants K_a values at various temperatures were obtained from the double reciprocal plot (List at Table 1). Thus, from the data of Table 1, the association constants were found to decrease gradually with increasing temperature. Using classical method of plotting lnK_a against $1/T$ (i.e., the Van't Hoff method) [26], see inset of Fig. 6, the corresponding enthalpy ΔH (−27.85 KJ mol^{-1}) and entropy ΔS (11.02 J mol^{-1} K^{-1}) are obtained from the slope and intercept of the graph (with 10 K intervals). The results suggest that the formation of complex with CB7 is favored by enthalpic factor, but small entropic driving force. In aqueous solution, water molecules are enclosed in the cavity of cucurbit [7] uril and additional water molecules are located at the carbonyl groups of the ligand [27, 28]. The removal of high energy water molecules from the host

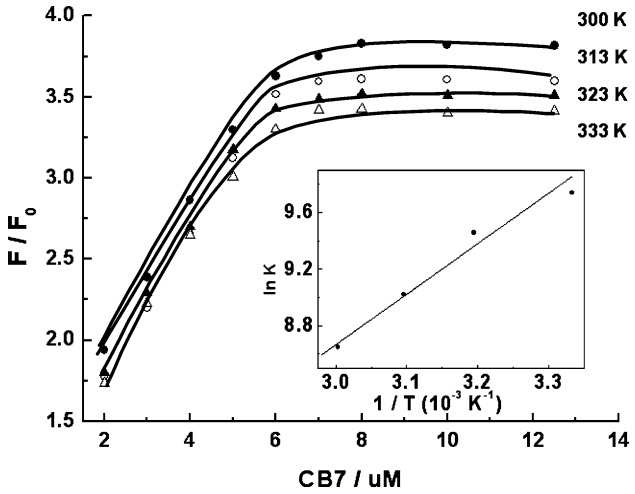


Fig. 6 Changes in the integrated fluorescence intensity vs. [*CB7*] for the complex at different temperatures. The inset shows the plot of ln *K* vs. $1/T$

Table 1 Inclusion constants of complexes at different temperature

Temperature (T, K)	Inclusion constants (K, M^{-1})	R
300	$K = 1.70 \times 10^4$	0.9991
313	$K = 1.28 \times 10^4$	0.9996
323	$K = 0.83 \times 10^4$	0.9983
333	$K = 0.57 \times 10^4$	0.9966

cavity and the desolvation of the cation only result in small enthalpic contributions [29]. The main contribution to the reaction enthalpies results from dipole–dipole interactions between the AO and the carbonyl groups of the CB7 and the solvent molecules are released during the complex formation. Similar observations were found in the complexation of organic cations with cucurbit [6] uril [29, 30] which indicated that the ion–dipole interactions between the protonated amino groups of amino acids and the carbonyl groups of CB6 are the important factor in the formations of the corresponding host–guest inclusion. The value of the relevant free energy change for this system at 298 K is $\Delta G = -31.15$ kJ mol^{-1} indicating an energetically favored reaction.

Influence of surfactants and comparison of the photophysical properties upon complexation with CB7 and β-CD

The surfactant can improve solubility of organic dye in water by changing the microenvironment and ipso facto influences physicochemical properties, e.g. binding constant [23]. Three kinds of surfactants: the cationic surfactant cetytrimethyammonium bromide (CTAB), the anionic surfactant

sodium dodecyl sulfate (SDS) and the non-ionic surfactant octylophenylpolyoxyethylene ether (Triton X-100) were chosen to study their effect on AO and CB7-AO complex. As is shown in Fig. 7, it is found that Triton X-100 has no effect on the fluorescence intensity of AO or the complex of AO-CB7 [31]. SDS with a negative charge can promote the formation of AO dimmer and the AO-CB7 complex being partially dissociated, therefore, the fluorescence intensity of AO in absence and presence of CB7 decreases obviously which accompany with a big wavelength shift [32]. Moreover, it can be seen that CTAB cannot affect the fluorescence intensity of AO, while the fluorescence intensity of AO-CB7 decreases obviously when appropriate amount of CTAB were added in. Since many organic cations can be included into the cavity of CB7 [10, 11], when the concentration of CTAB increases, the cationic surfactant has a competition to the CB7 with AO, which leads to the AO-CB7 complex being partially dissociated [33].

Cyclodextrins (CDs) are a well-known family of cyclic oligosaccharides consisting of six or more d-(+)-gluocopyranose units, with a hydrophilic outer surface and a hydrophobic internal cavity. The special cavity structure enables CDs to form guest–host inclusion complexes with both organic and inorganic compounds. Cyclodextrins have also been widely used to enhance the luminescence properties of different compounds [26, 34]. The cavity size of β-cyclodextrin is similar to the cavity of CB7 [35]. It is interesting to compare our results to those reported for AO in β-cyclodextrin (β-CD, see Chart 1). A 1:1 inclusion complex of AO by β-CD was reported with a lower value for the association constant; the fit value was 926 M^{-1} in β-CD [36], compared with the value of $(1.70 \pm 0.30) \times 10^4$ L mol^{-1} in CB7. Generally speaking, CB7 forms at least 100 times stronger inclusion complexes with AO than β-CD. Therefore we can say that CB7 is a much "better" host for acridine orange.

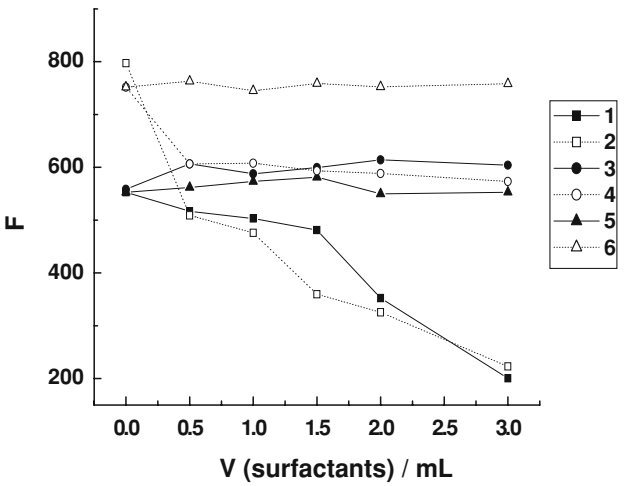


Fig. 7 Influence of surfactants concentration on the fluorescence intensity of CB7-AO system: (1) AO + SDS; (2) CB7 + AO + SDS; (3) AO + CTAB; (4) CB7 + AO + CTAB; (5) AO + TritonX-100; (6) CB7 + AO + TritonX-100; $C_{AO} = 5.00 \times 10^{-6}$ mol L^{-1}, $C_{CB7} = 2.00 \times 10^{-6}$ mol L^{-1}

Conclusion

In this work, Fluorescence spectrometry was applied to investigate the inclusion interaction of acridine orange with cucurbit [7] uril in aqueous solution. The absorption was accessorial to show the formation of complex. Experimental results indicate AO and CB7 form 1:1 host–guest inclusion complex. The stability constants of inclusion complexes were calculated by a deduced equation. Compared with the β-CD, the cucurbit [7] uril showed better inclusion capability. The probable interaction mechanisms were also discussed. It exhibits that cucurbit [7] uril and its inclusion compounds may have potential biological and medical applications like cyclodextrins although further research still need to be carried out.

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