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Host-guest complexation of melatonin with p-sulfonated calix[6]Arenes

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ABSTRACT

The complex characteristics of p-sulfonated calix[6] arene (SC6A) and Melatonin (MLT) were examined through spectrofluorimetry, ^{1}HNMR spectroscopy and molecular modeling calculations. The fluorescence of MLT significantly quenched upon the addition of SC6A, which revealed the formation of inclusion complexes between MLT and SC6A. The stoichiometric ratio of 1:1 was obtained via the continuous variation method. The experimental results show that SC6A forms at least 1000 times stronger inclusion complexes with MLT than β–CD and CB[7], which have the similar size of the cavity. The ^{1}HNMR spectra verified that the MLT may be partially penetrated into the hydrophobic cavity of SC6A. This finding was also confirmed by density functional theory calculations. The study is expected to provide important insight into the interactions of the physiologically important MLT with macrocyclic supramoleculars. It can also be as a fluorescence probe and sensor to detect non-fluorescent or weakly fluorescent substances.

KEYWORDS

Spectrofluorimetry;
p-Sulfonated calix[6]arene;
Cucurbit[7]uril;
β-Cyclodextrin;
Melatonin;
Molecular modeling computation.

INTRODUCTION

In recent years, inclusion complexation and molecular recognition have brought about a great deal of interest in the field of host–guest chemistry or supramolecular chemistry[1-3]. The importance of molecular recognition in various biological processes has helped chemists to design molecular systems with fascinating properties[4]. Calixarenes[5], the third generation of host suparmolecules, have attracted considerable attention in host-gust in the life sciences, materials science, pharmacology and supramolecular chemistry research show that wide application in molecular recognition and sensing as a result of their utility in rigid scaffolds[6]. Calixarenes are cup-shaped macrocycle hosts which are readily available via the condensation of a para-substituted phenol with formaldehyde[7,8]. Chemical modification of lower or upper calixarene rims by introducing groups with different binding abilities enables them to form inclusion complexes with a wide variety of guest species. Depending on the appended groups and the number of repeating phenolic units (usually four or six), which defines a macrocycle cavity size[9]. However, the poor solubility of calixarenes in aqueous solution limited their applications. These host molecules have a cavity-shaped structure that can hold a guest molecule and create specific affinity to a target molecule by introducing various functional groups[10]. Various functional groups, contain-

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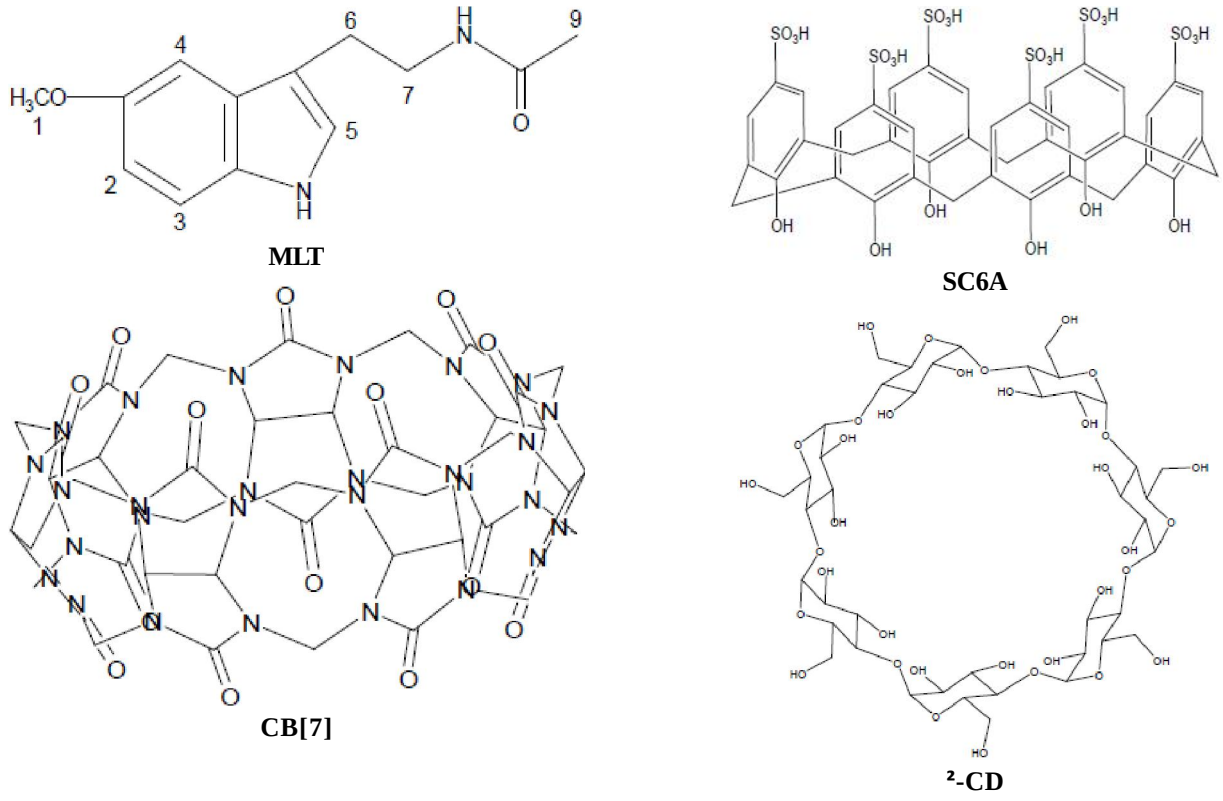

Scheme 1 : The structure of molecules are melatonin, p-sulfonated calix[6]arene, cucurbit[7]uril and β–cyclodextrin successively

ing dialkylamino groups, phosphonic acid groups, carboxyl groups, sulfonic groups and so on, could be introduced to either the upper or the lower rim of the 'cup', which could change the affinity of these cyclooligomers towards target molecules or increase the solubility of the calixarenes[11-13].

Studies have investigated p-Sulfonated calix[6]arene (SC6A) (scheme 1), which have flexible and often poorly defined cavities that bind positively charged species. SC6A are regarded as promising water-soluble hosts[14] for quaternary ammonium ions[15,16], trimethylammonium cations[17-19], dyes[20,21], native amino acids[22,23] and small neutral organic molecules[24]. Furthermore, SC6A have been applied in the improvement of solubility and stability of drugs and enzyme mimics[25-29].

Melatonin (MLT) (scheme 1) is well known as a paracrine hormone that is secreted in a cyclic manner by the pineal gland[30]. In mammals, the pineal gland is believed to be the major source of circulating melatonin[31]. It detoxifies a variety of free radicals (OH), peroxynitrite anion, singlet oxygen, and nitric oxide[32]. Melatonin controls biological rhythms, pigment metabolism, immune response, metabolism of free radicals, monitoring of mood and sleep, cell proliferation and differentiation[33]. MLT can perform as a valuable drug for prevention or cure of several diseases; its present breadth of applications, ranging from sleep-induction and antiageing action to cancer therapy, could be further extended by several clinical or pre-clinical studies in progress[34].

Over the past years, interactions between MLT and supramolecules or host–guest molecules have been widely studied. Ernane et.al[35], discovered a spectrofluorimetric method for the determination of MLT through the interaction with lipid bilayers. Hany et.al[36], investigated some new spectrofluorimetric methods for determination of MLT in the presence of N-{2-[1-({3-[2-(acetylamino)ethyl]-5-methoxy-1H-indol-2-yl}methyl)-5-methoxy-1H-indol-3-yl]-ethyl}acetamide: a contaminant in commercial melatonin preparations, MLT was determined in laboratory prepared mixtures containing different percentages of compound. However, to the best of our knowledge, the supramolecular interactions between

SC6A and MLT were determined by spectrofluorometry have not been reported.

In our experiment, MLT have highly fluorescent nature and efficient fluorescence quenching property upon binding with SC6A by fluorescence titrations, A gradual decrease in the fluorescence intensity was observed with the SC6A concentration increased. Temperature-dependent inclusion constants were also obtained, ^{1}HNMR spectra and molecular modeling analyses were performed to investigate the possible mechanism of the binding reaction.

Moreover, a comparative study of the complexation behavior of fluorescent MLT with cucurbit[7]uril (CB[7]) and β-Cyclodextrin (β–CD) (scheme 1) has been carried out on the basis of results obtained by spectrofluorimetry, ^{1}HNMR spectroscopy and molecular modeling calculations.

EXPERIMENTAL

Apparatus

Fluorescence spectra and measurements were obtained using a Agilent Technologies Cary Eclipse Fluorescence spectrofluorometer equipped with 150 W xenon lamp (Japan). The slit widths of both the excitation and emission monochromators was set at 5 nm. The fluorescence spectra were recorded at a scan rate of 600 nm min^{-1}. All measurements were performed in a standard 10 mm path-length quartz cell set to a temperature of 25.0±0.5°C. The pH values were measured using a pHS-3TC digital precision pH meter (Shanghai, China). In the experiment, the temperatures were controlled by using a thermostated cell holder and a thermostatically controlled water bath. ^{1}HNMR spectra was recorded using a Bruker DRX-600MHz spectrometer (Switzerland). Molecular modeling calculations were optimized at the B3LYP/6-31G(d) level of density functional theory with the Gaussian 03 program.

Reagents

All reagents used were of analytical reagent grade or the best grade available commercially, and double-distilled water was used throughout the procedures. The MLT used in the experiment were obtained from the National Institute of Metrology (China). Its purity was 99.5%. The stock solution of 1.0×10^{-3} mol L^{-1} was prepared by directly dissolving in double-distilled water. SC6A was purchased from TCI Chemical Industry Co, Ltd (Shanghai, China). SC6A stock solution of 1.0×10^{-3} mol L^{-1} were prepared respectively in a 100 mL volumetric flask. β-Cyclodextrin (Sinopharm Chemical Reagent Shanghai Co., Ltd.) was recrystallized twice from water and dried under a vacuum at 95 ºC for 24 h prior to use. CB[7] was prepared and characterized according to recently reported procedures[37]. Working solutions were obtained by dilution of the stock solution. The standard MLT solution was found stable over time during the experiment, its fluorescence intensity was not change. The stock solutions were kept at 4°C degree. The working solution was prepared freshly. The SC6A stock standard solutions were stable for several weeks at room temperature. A Britton-Robinson (BR) buffer solution (pH 2.00–12.00) was prepared using 0.04 mol L^{-1} boric acid, acetic acid and phosphoric acid, and then was adjusted to accurate values by using 0.2 mol L^{-1} sodium hydroxide.

Experimental procedure

A total of 1.0 mL of 1.0×10^{-4} mol L^{-1} MLT solution was transferred into a 10 mL volumetric flask, and an appropriate amount of 1.0×10^{-4} mol L^{-1} SC6A was added. The pH was controlled by 1.0mL of Britton-Robinson buffer solutions. The mixed solution was diluted to the final volume with distilled water and shaken thoroughly, then equilibrated for 15 min at room temperature. The fluorescence intensity values of the experimental and blank solutions (F_{MLT}) were measured using at 358 nm an excitation wavelength of 227 nm.

RESULTS AND DISCUSSION

Fluorescence quenching of the SC6A-MLT complex

Aqueous solutions of MLT have strong fluorescence in aqueous solution and the maximum excitation and emission wavelengths were located at 227 and 358 nm, respectively. However, addition of SC6A brought a markedly decrease in MLT fluores-

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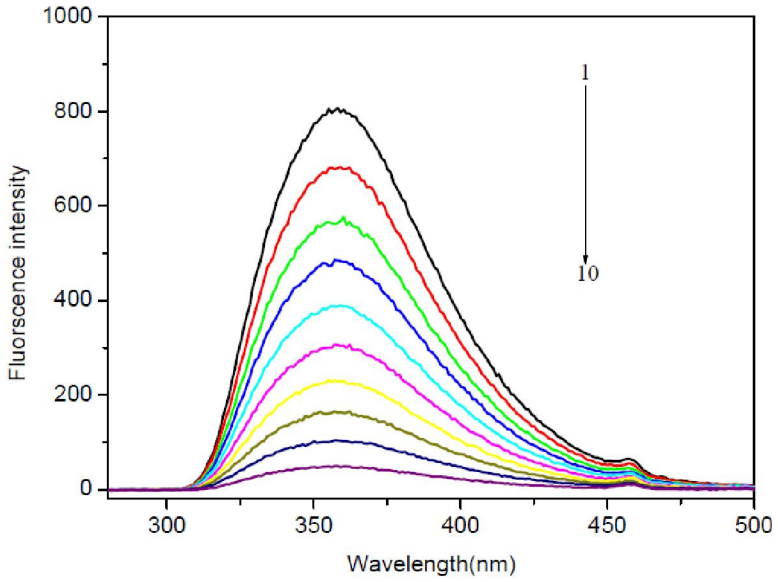

Figure 1 : The fluorescence spectra of MLT in different concentrations of SC6A in britton–robinson buffer solutions. The concentrations of SC6A(10^{-4}mol L^{-1}) (1) 0; (2) 0.3; (3) 0.5; (4) 0.7; (5) 0.9; (6) 1.0; (7) 1.2; (8) 1.5; (9) 1.7; (10) 2.0; C_{MLT}=1.0×10^{-4}mol L^{-1}

cence intensity. These modifications of the features of the fluorescence spectra were considered to be a result of inclusion complex formation between MLT and SC6A.

An interaction between MLT and SC6A was confirmed by flurescence spectra when the initial concetration of MLT was 1.0×10^{-4} mol L^{-1}. Figure 1 shows the fluorescence spectra of MLT in the absence and presence of SC6A. Fluorescence intensity gradually decreased with increased SC6A concentration.

The temperature and pH of the experiment were optimized. In Britton–Robinson buffer solutions with pH 7.4 and at room temperature, the fluorescence of MLT was significantly quenched upon a certain amount of SC6A. Therefore, a pH of 7.4 was used for all subsequent experiments and room temperature was selected as the standard reaction condition.

Stoichiometry and association constant of the inclusion complex

Inclusion capacity of a host in relation to specific guest was described as the inclusion formation constant (K). Under the optimum experimental conditions, with 1:1 MLT-SC6A complex, K could be obtained by the following calculation:

SC6A+MLT⇔SC6A-MLT **(1)**

$$K = \frac{C_{SC6A\text{-}MLT}}{C_{SC6A}\cdot C_{MLT}} \quad \textbf{(2)}$$

Where C_{MLT}, C_{SC6A}, and $C_{MLT–SC6A}$ are presented as equilibrium concentrations. Thus, K value can be determined by the typical double reciprocal or Benesi-Hildebrand plots[38].

$$\frac{1}{F-F_0} = \frac{1}{(F_\infty - F_0)KC_{SC6A}} + \frac{1}{F_\infty - F_0} \quad \textbf{(3)}$$

Where F is the observed fluorescence intensity when each corresponding SC6A concentration was tested, F_0 is the MLT fluorescence intensity without SC6A addition, and F_∞ is the enhancement once all MLT complexs are formed.

A good linear relationship was obtained when $1/(F-F_0)$ was plotted against $1/C_{SC6A}$, which supports the existence of a 1:1 complex(Figure 2). The binding constants of the MLT and SC6A complexes at pH 7.4 were determined to be 4.97×10^4 M^{-1} in the presence of SC6A. The value was determined by dividing the intercept by the slope of the Y corresponding lines.

Generally, the binding stoichiometry of MLT with SC6A was determined by Job's plots and was found to be 1:1(Figure 3). The maximum of the relative fluorescence intensity was at a mol fraction of 0.5, which confirmed the formation of a 1:1 ratio com-

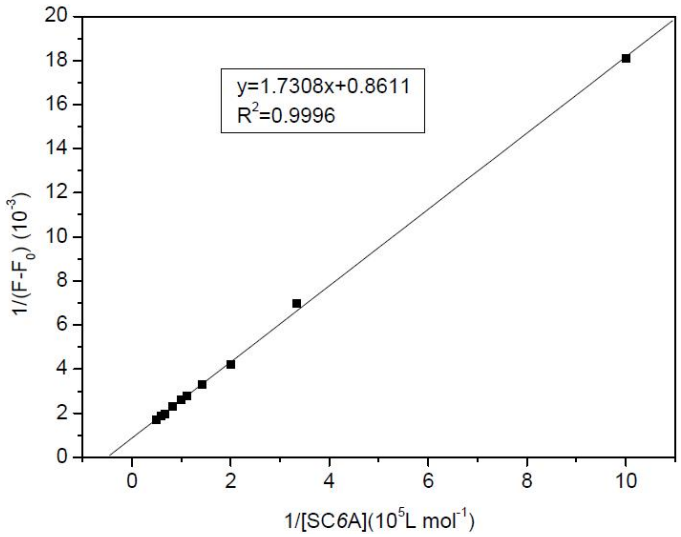

Figure 2 : The relationship of $(F-F_0)^{-1}$ with $SC6A^{-1}$, T=298K, C(MLT)=10^{-4}M, pH=7.4

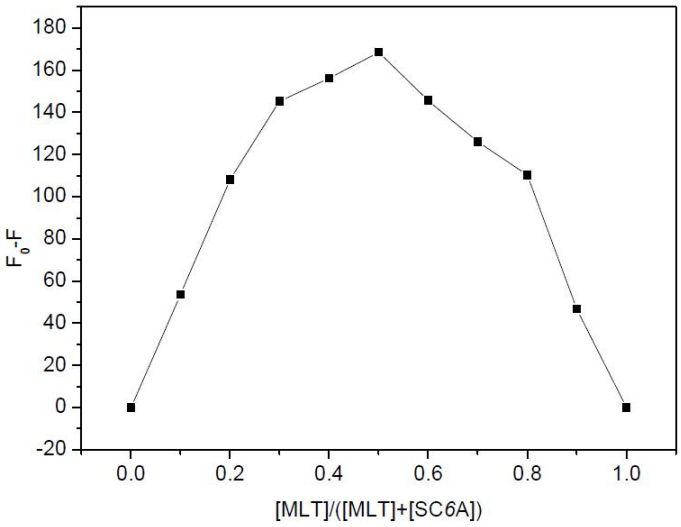

Figure 3 : Job's plot for the complex of MLT with SC6A in Britton-Robinson buffer solution (pH 7.4) at 25!.([MLT]+[SC6A])=1.0×10^{-5} mol L^{-1}

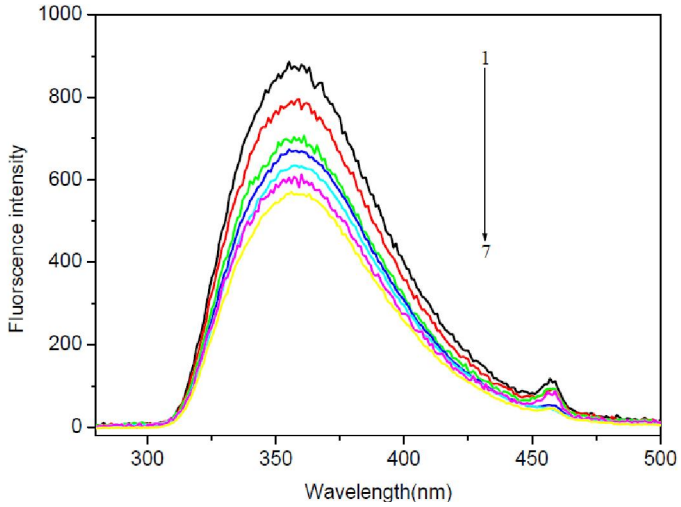

Figure 4 : The fluorescence spectra of MLT in different concentrations of β–CD in Britton–Robinson buffer solutions. The concentrations of β–CD (10^{-4} mol L^{-1}) (1) 0; (2) 0.5; (3) 1.0; (4) 1.5; (5)2.0; (6) 3.0; (7) 4.5; C_{MLT}=1.0×10^{-4}mol L^{-1}

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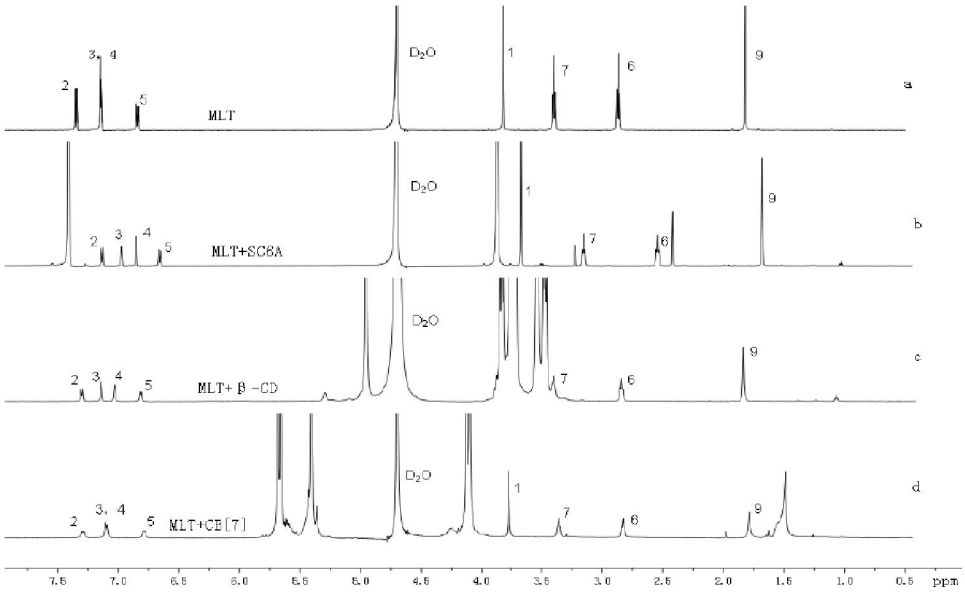

Figure 5 : ^{1}HNMR spectra (600 MHz) of (a) MLT (b) SC6A-MLT complex (c) β–CD-MLT complex and (d) CB[7]-MLT complex in D_2O

plex[39].

^{1}HNMR spectroscopy and molecular modeling calculation

The formation of SC6A-MLT inclusion complexes in aqueous solution was confirmed using ^{1}HNMR Spectroscopy(Figure 5b). Compared with the proton resonances of the unbound MLT molecules (Figure 5a), the signals from H1, H2, H3, H4, H5, H6, H7, and H9 protons of the bound MLT significantly shifted upfield. Especially, the singlets for H3, H4 protons inside the SC6A cavity should be sensitive to the changed environment after the complexation were observed clearly. This behavior is characteristic of this part of the MLT molecule encapsulated in the SC6A cavity. Furthermore, several protons chemical shifts of MLT were changed. It confirmed that the complex was formed. These results are consistent with the previous fluorescence quenching.

Molecular modeling calculations were optimized at the B3LYP/6-31G[40] lever of density functional theory[41,42] using the Gaussian 03 program[43]. Molecular mechanics was simulated to obtain the optimized conformation of the host-guest complex (Figure 6). The SC6A-MLT molecular models are shown in Figure 6a. In the energy minimized structure, the complexation reactions MLT with SC6A are very sensitive since the high flexibility of SC6A.

It is well known that the complexation between the guest and the calixarene are formed by weak forces including hydrogen bonding, p–p interaction, electrostatic interaction, hydrophobic interaction, dipole–dipole or van der Waals[44]. Generally, in the process of the formation of the inclusion complex, the key force depends on the structure, the charge, functional group of guest and host. SC6A has an upper laced with six hydroxyl groups and a socially wider rim with modified sulfonic groups, and indole benzene ring of MLT is located in the vicinity of a carbonyl-laced portal. The formation of hydrogen bond between hydrogen at N atom of indole ring and the sulfonic groups may cause the changes of the geometric configuration of indole ring. The carbanyl group and hydroxy of SC6A may form hydrogen bond. The NH of aliphatic chain is likely to form hydrogen bond with the hydroxy of SC6A. Hydrogen bonding interaction and the electrostatic interaction lead to the formation of host-guest inclusion complex. This state results in a less polar microenvironment which, in turn, leads to fluorescence quenching. These results are consistent with the foregoing discussion.

Comparison of the Photophysical Properties upon Complexation with SC6A, CB7 and β–CD.

David et.al[45]. obtained the 1:1 complex formation between MLT and β–CD from the ^{1}HNMR spec-

troscopy, calorimetric and solubility measurements, and mass spectrometry. The inclusion constants for these 1:1 complexes were determined to be 48.0 M^{-1} in the presence of β–CD. The point to note here is that the binding constant significantly less than the binding constants of the SC6A inclusion complexes. In our study, the supramolecular interaction between MLT and β–CD has been studied using fluorometric method. Figure 4 showed the fluorescence spectra of the MLT in the absence and presence of β–CD. The fluorescence intensity of the MLT significantly decreased upon the addition of β–CD. The fluorescence quenching of SC6A was more significant than that of β–CD at the same MLT concentration. Meanwhile, we examined the interaction of CB[7] with MLT by fluorometric method and the system has no the effect of fluorescence change. The mechanisms of the inclusion process of MLT with β–CD and CB[7] have also been discussed by the ^{1}HNMR and molecular modeling calculation.

A rough estimation of the hydrodynamic molecular geometric dimensions can be obtained by considering the complex as an effective sphere and the hydrodynamic diameter similar to the inside diameters of the SC6A (0.76 Å), CB[7] (0.73 Å) and β–CD (0.78Å). The formation of SC6A-MLT, CB[7]-MLT, and β–CD-MLT inclusion complexes in aqueous solution was confirmed using ^{1}HNMR spectroscopy experiments were carried out in D_2O at room temperature. Figure 6 also shows the ^{1}HNMR spectra of a 1:1 host-guest complex betweeen MLT and the two macrocyclic host molecules.

Compared with the proton resonances of the free MLT molecules(Figure 5a), the chemical shift of MLT protons changed after complexation with macrocyclic host molecules (Figure 5). The signals from H3 and H4 protons of the bound β–CD significantly shifted upfield, this behavior is characteristic of this part of the indole benzene ring of the MLT molecule encapsulated in the β–CD cavity. The chemical shift of the H1, H2, H5, H6, H7 and H9 protons of the β–CD-MLT is practically unchanged, which indicates the protons of this part of the molecule located just outside the cavity of the β–CD host. The resonance of protons H3 of CB[7]-MLT experienced a slightly up-field shift, indicating this part of the molecule was located just inside the carbonyl portal of the CB[7] host. The chemical shift of the H2, H3, H4, H5, H6, H7 and H9 protons of the CB[7]-MLT is practically unchanged, indicating this part of the molecule was located just outside the carbonyl portal of the CB[7] host.

The MLT molecules immersed in the cavity of SC6A, which is expected to enter CB[7] and β–CD cavity. The result confirmed the partial inclusion of MLT in the hydrophobic cavity of β–CD, and CB[7] using the Gaussian 03 program, Respectively(Figure 6). The restriction of the conformational mobility is not compensated by the release of water molecules from the MLT molecules and CB[7] a slight rise in the chemical shift is observed for the weaker-bound MLT-CB[7] complexes, hydrogen bonds[46] and electrostatic interactions between both molecules are weak. The polar carbonyl groups at each portal of CB[7] and their simultaneous interactions with the protonated amino groups are responsible for this effect. The NH of aliphatic chain is located in the vicinity of a carbonyl-laced portal, the methoxy group is located in the vicinity of the other carbonyl-laced portal. The partial immersion of MLT in the hydrophobic cavity via portals of CB[7] attributed to hydrogen bonding. The hydroxyl groups located at both rims of β-CD are likely to form hydrogen bonds with the amino groups and the NH of the indole ring. Hydrogen bonding interaction may lead to the formation of host-guest inclusion complex. Because of the excellent matched size and morphology, benzene ring residues were enclosed in the cavity of β–CD more tightly than CB[7] in aqueous solution. These results are consistent with the trend observed in ^{1}HNMR spectra and the previous fluorescence phenomenon.

Generally speaking, SC6A forms at least 1000 times stronger inclusion complexes with MLT than β–CD and CB[7]. For potential practical applications, it is important to note that a virtually quantitative complexation of MLT can be readily achieved even with small amounts of SC6A. This is due to the high binding constants. From a photophysical point of view, the addition of both host molecules results in a fluorescence quenching of MLT. Because of the more flexible conformation of SC6A >β–CD>

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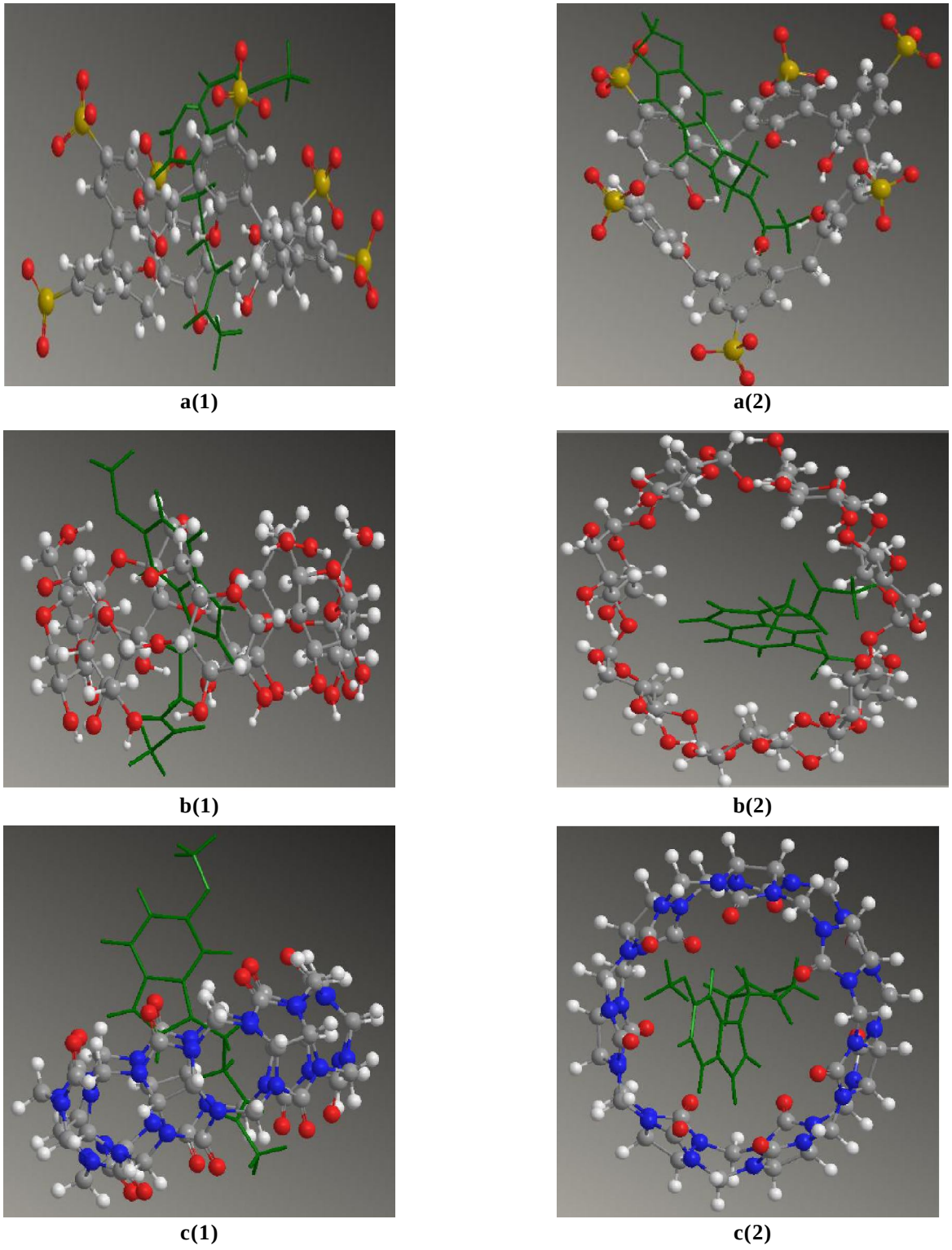

Figure 6 : Lowest energy structure of complex using ball and stick model determined by molecular dynamics simulation with direct minimization for the rendering of atoms. (a) MLT and SC6A complex, (b) MLT and β–CD complex, (c) MLT and CB[7] complex. (1) profile (2) above

CB[7], the hydrophobic segments of the benzene ring of SC6A may be the most easily adsorbed to MLT molecule. (see Figure 6). The dramatic fluorescence quenching provides strong evidence that inclusion complexes are indeed formed between MLT and SC6A.

CONCLUSIONS

The results obtained from the spectrofluorimetric studies indicate that both SC6A form inclusion complexes with MLT, particularly strong for the interaction MLT-SC6A complex ($K>10^4$ M^{-1}). According to the data, SC6A can react with MLT to from 1:1 inclusion complex. The association constants of the complexes formed between the host and the guest were calculated. The interaction mechanism was confirmed via the ^{1}HNMR spectrum. The interaction models of the supramolecular complexes were established through theoretical calculations. The comparsion of the complexation behavior of MLT toward SC6A with that toward β–CD and CB[7] reveals that although all hosts have a simliar hydrophobic cavity, additional recognition elements such as sulfonic groups receptor sites at the portals of the cavity are quintessential to promote a strong and selective binding of MLT, which may affect the fluorescence intensity of MLT.

The possible complexation mechanism for MLT and SC6A may involve hydrophobic and electrostatic interaction. This study can be used as a fluorescence probe and sensor to detect non-fluorescent or weakly fluorescent substances. This work will be helpful to provide useful information for appropriately understanding of the drug design and pharmaceutical research. Related studies are currently underway.

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