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Interactions of New bis-Ammonium Thiacalix[4]arene Derivatives in *1,3-Alternate* Stereoisomeric Form with Bovine Serum Albumin

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Abstract New bis-ammonium thiacalix[4]arene derivatives with different lipophilicity were synthesized using copper(I)-catalyzed azide-alkyne cycloaddition in good yields. Binding of a new thiacalix[4]arene derivatives with bovine serum albumin (BSA) was investigated in detail using fluorescence spectroscopy method. Quenching mechanism, the binding constants, and number of binding sites were determined.

Keywords Thiacalix[4]arene · Triazoles · Ammonium compounds · BSA · Luminescence · Quenching of luminescence

1 Introduction

Proteins are very important biomacromolecules due to their relation to alimentation, immunity, and metabolism of mammals. Serum albumins are the most abundant proteins in blood, synthesized and secreted from liver cells [1, 2]. They have many important physiological functions. For instance, they contribute to the osmotic blood pressure and are chiefly responsible for the maintenance of blood pH. But the most important physiological role of albumins involves the binding, transport, and delivery of a variety of endogenous and exogenous substances [3]. The structure of BSA molecule is similar to human serum albumins (HSA) molecule in 76 %; thus, BSA molecules are usually used as model of HSA [4].

Calix[n]arenes and their thia-analogues are well-known macrocyclic compounds having a unique basket shape [5]. Calix[n]arenes have been widely used to construct different supramolecular receptors, host-guest complexes, and components of molecular devices due to their variety of stereoisomeric forms and facile functionalization of both upper and lower rims [6, 7]. It is well-known that BSA can effectively bind anionic molecules [1]. There are several publications comprising interactions of anionic (carboxylic or sulfonate) calixarene derivatives with BSA [8, 9]. However, for the best of our knowledge, there is lack of publications devoted to study of interactions between cationic calixarene derivatives with BSA although it is known that BSA is negatively charged at physiological conditions which may contribute to bind cationic molecules both by electrostatic and hydrophobic forces.

2 Material and Methods

Synthetic procedure of compounds **1**–**3** is described in supplementary information.

Fluorescence experiments were performed in 1 cm quartz cuvettes and recorded on a Fluorolog FL-221 spectrofluorimeter (HORIBA Jobin Yvon) in the range of 290 to 450 nm and excitation wavelength 280 nm with 3 nm slit for the BSA. All studies were conducted in buffered aqueous solution (TRIS buffer, pH 7.4) at 298.

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3 Results and Discussion

The corresponding tetra-substituted thiacalix[4]arene azides with alkyl groups of different length were synthesized by stepwise Mitsunobu reaction with alkyl alcohols on the first step and 3-bromopropanol-1 at the second with following bromine substitution by azido-group as described previously [10]. bis-Ammonium thiacalix[4]arene derivatives were synthesized by CuACC reaction with *N*-propargyl-*N*,*N*,*N*-triethylammonium bromide.

Fluorescence quenching of the protein could be used to retrieve information about many substrate-protein binding interactions. The BSA fluorescence is practically composed of the tryptophan emission due to two Trp residues (Trp-212 and Trp-134). Trp-134 is located on the surface of BSA molecule while Trp-212 is located within a hydrophobic pocket of BSA [11]. As could be seen in Fig. 1, SI, the intrinsic fluorescence of BSA molecules at 331 nm could be greatly quenched by thiacalix[4]arene **1**–**3** derivatives. The fluorescence quenching is usually divided into dynamic and static one. Dynamic quenching is the result in the collisional encounters between the fluorophore and quencher, while static quenching is related to the formation of a ground-state complex between the fluorophore and quencher [12] (Fig. 2).

Fluorescence quenching is described by the Stern-Volmer Eq. (1).

$$F_0/F = 1 + K_{sv}[Q] = 1 + K_q\tau_0[Q] \quad (1)$$

where K_{sv} and $[Q]$ are dynamic Stern–Volmer quenching constant and quencher concentration, respectively; F_0 and F are the fluorescence intensities in the absence or presence of quencher, respectively; K_q is the biomolecular quenching constant; and τ_0 is the average lifetime of protein in the absence of quencher and its value about 10^{-8} s [12]. Stern–Volmer quenching constant (K_{sv}) can be obtained from linear regression of a plot of F_0/F against $[Q]$. In the case of calixarene **3**, both dynamic and static quenching is involved because the Stern–Volmer plots deviated from linearity toward the *Y*-axis at high calixarene concentration. Fluorescent titration of BSA by calixarene **3** at the different temperatures showed that temperature increase leads to the decrease of the graph slope indicating that static quenching is predominant [13]. Moreover, the K_q of 1.22×10^{12} M^{-1} s^{-1} for calixarene **3** is much more than the value of the maximum diffusion collision quenching rate constant of 2.0×10^{10} M^{-1} s^{-1} [12]. Therefore, the quenching of BSA fluorescence by calixarenes **1**–**3** is mostly static quenching process due to the complex formation between quencher and protein. In this case, the binding constant can be determined from the fluorescence intensity data using Eq. (2) [14].

$$\lg(F_0 - F)/F = \lg K_a + n\lg[Q] \quad (2)$$

where n is the number of binding sites, K_a is the binding constant of calixarene with BSA, which can be determined by the slope of double logarithm regression curve of $\lg(F_0 - F)$ versus $\lg[Q]$. The corresponding values of $\lg K_a$ and n are presented in Table 1.

According to the obtained data the calixarene lipophilicity increase especially in the case of compound **3** results in 4-order growth of binding constant K_a. It is noteworthy that number of binding sites (n) also increases with lipophilicity going from 1.2–1.3 in the case of compounds **1**, **2** to 2.0 in the case of compound **3**. This can be better explained as follows: interactions of BCA with butyl and octyl calixarene

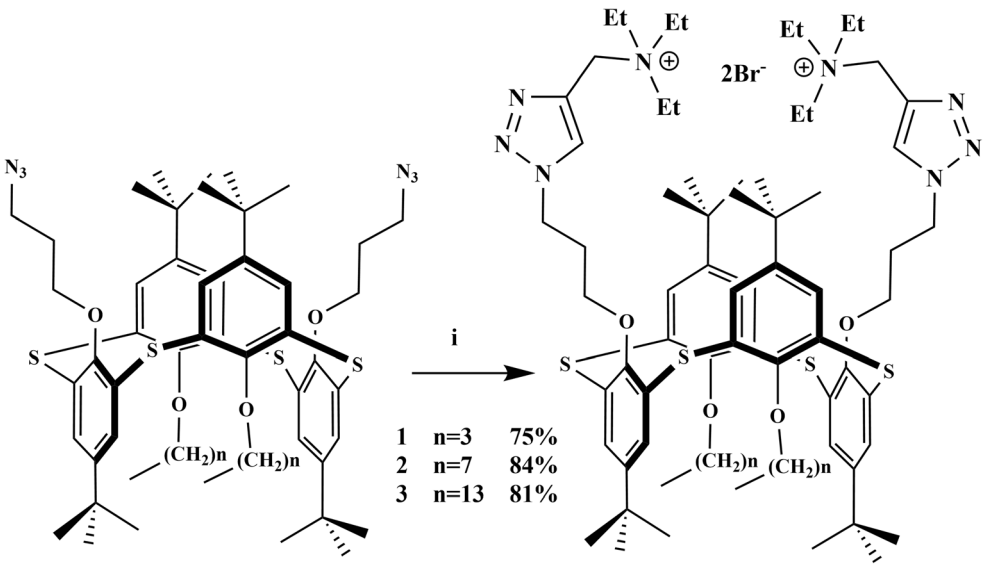

Fig. 1 Reaction conditions: i–$CHCCH_2N(Et)_3Br$, CuI, DMF-toluene, 40 °C

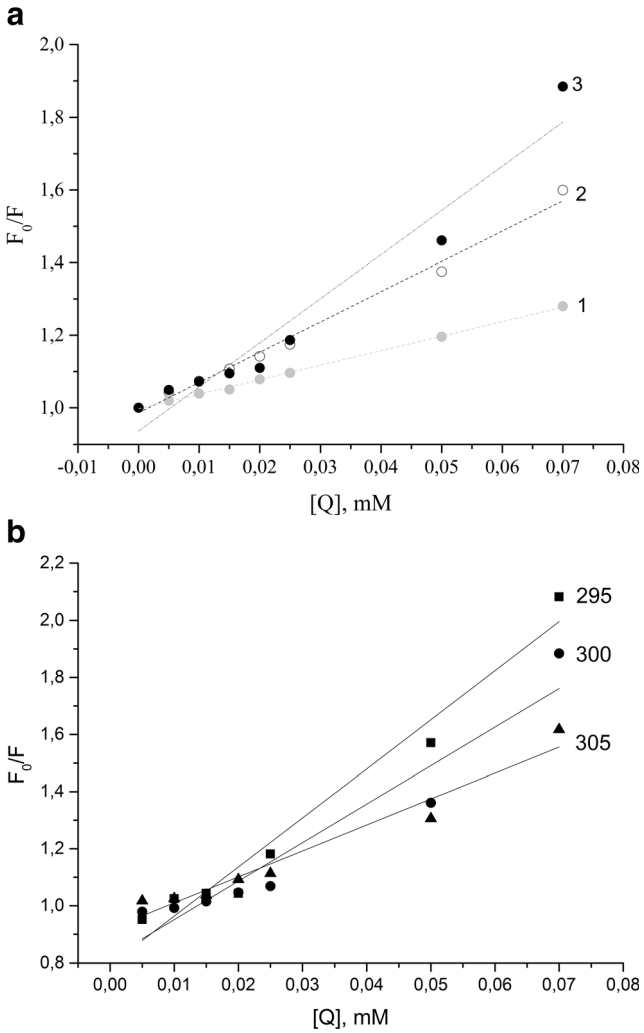

Fig. 2 Stern–Volmer plots of fluorescence quenching of BSA by calixarenes **1**–**3** (**a**), Stern–Volmer plots of fluorescence quenching of BSA by calixarene **3** at different temperature (**b**), (C[calix] from 0 to 0.07 mM, C[BSA] = 0.01 mM, C[Tris–HCl] = C[NaCl] = 50 mM, pH = 7.4)

derivatives **1** and **2** occurs only through electrostatic interactions with Trp-134, located on the surface of BSA which corresponds to 1:1 stoichiometry. Increase of lipophilicity in the case of calixarene **3** results in the binding with hydrophilic surface of BSA through electrostatic interactions and with the hydrophobic binding pocket of BSA (sub-domain IIA, comprising Trp-212 residue) through hydrophobic interactions.

Table 1 Stern-Volmer quenching constant (K_{SV}), logarithm of binding constant (lgK_a), binding sites number (n), and correlation coefficient (R) of calixarenes **1**–**3** with BSA at 295 K

Calixarenes	$K_{SV} \times 10^3$*l/mol	R	lgK_a	R	n
1	3.65 ± 0.36	0.997	4.67 ± 0.22	0.992	1.2 ± 0.1
2	7.78 ± 0.48	0.989	4.85 ± 0.34	0.990	1.3 ± 0.1
3	12.21 ± 0.91	0.962	8.45 ± 0.44	0.985	2.0 ± 0.1

4 Conclusions

Binding of a new bis-ammonium thiacalix[4]arene derivatives with BSA was investigated in detail using fluorescence spectroscopy method. The experimental results showed that the fluorescence quenching of BSA by calixarenes was a result of the formation of complex between them and intrinsic fluorescence of BSA is reduced through a static quenching. The binding constants and number of binding sites were determined, and it was found that increase of calixarene lipophilicity gives more strong binding with BSA.

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