

Determination of binding constants of various alkali metal cations with *p*-sulfonatocalix[8]arene by using inclusion equilibrium with thionine dye

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Received: 23 February 2012 / Accepted: 8 June 2012 / Published online: 26 June 2012
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Abstract The binding constants of various alkali metal cations with water-soluble *p*-sulfonatocalix[8]arene (Calix-S8) were determined spectrophotometrically by using the inclusion equilibrium of thionine (Th) dye as a chemical indicator. Depending on the kind of alkali metal cations, the inclusion constants of Calix-S8 for Th decrease steeply with an increase in salt concentrations. Alkali metal cations compete with the organic guest Th in the Calix-S8 inclusion. Based on a competitive binding experiment, the binding constants of alkali metal cations with Calix-S8 were evaluated to be 17, 60, and 11 dm⁶ mol⁻² for Na⁺, K⁺, and Cs⁺, respectively. We have demonstrated an absorption-based method of evaluating the binding constants of spectroscopically inert metal cations with Calix-S8 and shown the effects of salts on the molecular recognition of Calix-S8.

Keywords Water-soluble *p*-sulfonatocalix[8]arene · Inclusion complex · Binding constant · Alkali metal cations · Phenothiazine dye

Introduction

Calixarene derivatives are attractive hosts for inclusion chemistry [1–3]. In particular, the water-soluble calixarenes

are powerful receptors for a variety of organic compounds and offer many possibilities for use in a wide variety of medical, biological, and industrial applications.

Calixarenes are reported to have significant ionophoric activity [4–10]. Using a fluorescence probe as a guest, Bakirci et al. [11] determined the binding constants of metal cations with *p*-sulfonatocalix[4]arene (Calix-S4) through a competitive binding experiment. Recently, Bisilio et al. [12] qualitatively detected the interactions between metal cations and *p*-sulfonatocalixarenes from ²³Na diffusion NMR (DOSY) measurements. In the inclusion of calixarenes, since metal cations can be complexed with calixarenes, the cations will compete with other guest molecules. Therefore, the qualification of metal cation binding constants is important.

The determination of binding constants for complexations using spectroscopic measurements such as absorbance is commonly accomplished by the Benesi–Hildebrand method [13]. The Benesi–Hildebrand analysis requires a spectroscopic change in absorption upon complex formation. Since calixarenes are spectroscopically inert in the visible region, spectroscopically active guests should be used. However, this constraint often leads to experimental difficulties. When spectroscopically active molecules are used as chemical indicators for the determination of binding constants, one can extensively evaluate the binding constants for calixarene complexation with molecules that do not exhibit a color change on forming a complex.

In this paper, we have reported an absorption-based method of evaluating the binding constants of alkali metal cations with water-soluble *p*-sulfonatocalix[8]arene (Calix-S8). As a chemical indicator, the phenothiazine dye, thionine (Th), which is spectroscopically active in the visible region, has been employed. The binding constants of three kinds of alkali metal cations with Calix-S8 were quantified

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for the first time. Based on the results, the molecular recognition of Calix-S8 for alkali metal cations is discussed.

Experimental

Materials and spectral measurements of inclusion complex

The phenothiazine dye, thionine (Th), and water-soluble *p*-sulfonatocalix[8]arene (Calix-S8) used in this study are shown in Fig. 1. Thionine was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and was recrystallized from an ethanol–water mixture and dried under vacuum before use. Calix-S8 was obtained from Sugai Chemie Inc. (Wakayama, Japan) and was recrystallized from an ethanol–water mixture. Reagent grade methanol was obtained commercially from Wako Pure Chemicals (Osaka, Japan).

We used a water–methanol mixture (1:1 (V/V) phosphate buffer pH 6.9, ionic strength = 0.01) as a solvent to avoid dimerization of Th. Under these condition, we have assumed that the buffer agents are not complexed by Calix-S8 because of the weak binding of the cations (see below). The spectral change of Th (2.49×10^{-5} mol dm⁻³) containing different concentrations of Calix-S8 (0 – 2.0×10^{-3} mol dm⁻³) in a water–methanol mixture in an excess of alkali salts (NaCl, KCl, and CsCl (0.1 – 0.8 mol dm⁻³: [Salts] \gg [Calix-S8])) was monitored by using a Hitachi U-3200 spectrometer (Tokyo Japan). The reaction temperature was controlled at 298 ± 0.1 K by circulating temperature-controlled water.

Results and discussion

Inclusion complexation of Th with Calix-S8

The absorption spectra of the phenothiazine dye, thionine (Th), upon consecutive addition of Calix-S8 in great excess of alkali salts (KCl) are shown in Fig. 2. Upon addition of Calix-S8 to a solution of Th in a mixture of methanol and

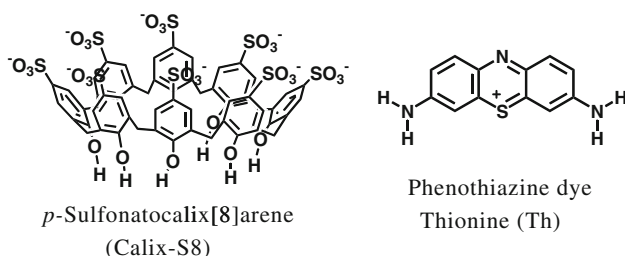


Fig. 1 Structures of phenothiazine dye Th and *p*-sulfonatocalix[8]arene

water, the peak in the vicinity of 600 nm caused by Th decreased. In previous papers [14, 15], based on UV–Vis and NMR studies, we have suggested that Calix-S8 forms a 1:1 inclusion complex with Th dye and proposed the structure of the inclusion complex.

The 1:1 inclusion equilibrium ($K(\text{Th})$) in the presence of excess of Calix-S8 ($[\text{Calix-S8}] \gg [\text{Th}]$) can be expressed as follows:

$$\begin{aligned} \text{Calix-S8} + \text{Th} &\overset{K(\text{Th})}{\rightleftharpoons} \text{Calix-S8} - \text{Th}, \\ K(\text{Th}) &= \frac{[\text{Calix-S8} - \text{Th}]}{[\text{Calix-S8}][\text{Th}]} \\ &= \frac{[\text{Calix-S8} - \text{Th}]}{[\text{Calix-S8}]_0([\text{Th}]_0 - [\text{Calix-S8} - \text{Th}])}, \end{aligned} \quad (1)$$

where Calix-S8 – Th denotes the inclusion complex. The symbol []₀ is the initial concentration. The spectral data were analyzed according to the Benesi–Hildebrand–Scott equation [13]. Because the concentration of Calix-S8 – Th can be given by $[\text{Calix-S8} - \text{Th}] = \Delta E / \Delta \epsilon$ (optical path length = 1), Eq. 1 can be rewritten as:

$$\frac{[\text{Calix-S8}]_0}{\Delta E} = \frac{1}{\Delta \epsilon [\text{Th}]_0} [\text{Calix-S8}]_0 + \frac{1}{\Delta \epsilon [\text{Th}]_0 K(\text{Th})}, \quad (2)$$

where $\Delta \epsilon$ is the difference in the molar extinction coefficients for bound and unbound Th with Calix-S8 and ΔE is the change in the absorption intensity (extinction) of the Th

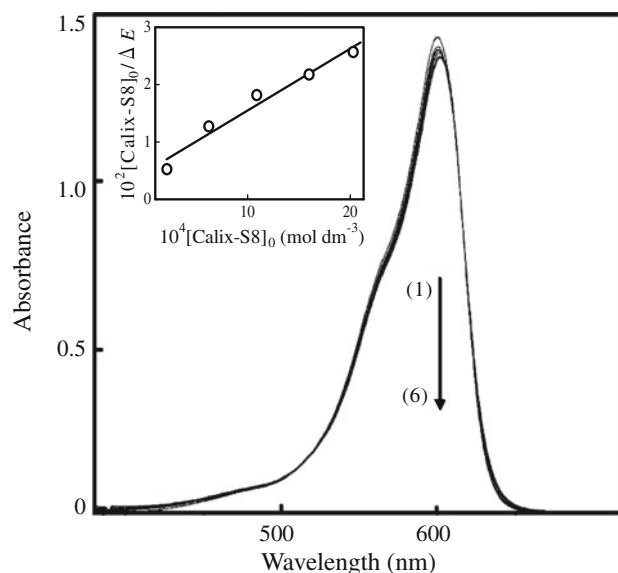


Fig. 2 Absorption spectra of Th (2.49×10^{-5} mol dm⁻³) containing different concentrations of Calix-S8 in a water–methanol mixture in excess of KCl (0.30 mol dm⁻³): [Calix-S8] = 1 0, 2 2.15×10^{-4} , 3 6.23×10^{-4} , 4 1.09×10^{-3} , 5 1.60×10^{-3} , and 6 2.03×10^{-3} mol dm⁻³. (inset) Determination of 1:1 inclusion equilibrium constant according to Eq. 2

Table 1 The inclusion equilibrium constants (K_{obs}) of Th in excess of alkali salts and the binding constants of alkali metal cations with Calix-S8 in a water–methanol mixture (1:1 (v/v)) at 298 K

Salt	Salt concentration (mol dm ⁻³)	$10^{-3} K_{\text{obs}}$ (dm ³ mol ⁻¹)	$K(M_2^+)$ (dm ⁶ mol ⁻²)
–	–	14.5 ± 0.7^a	–
NaCl	0.50	2.73 ± 0.12	17
	0.80	1.32 ± 0.05	
KCl	0.30	2.28 ± 0.18	60
	0.63	1.29 ± 0.07	
CsCl	0.50	3.88 ± 0.20	11
	0.80	1.79 ± 0.06	

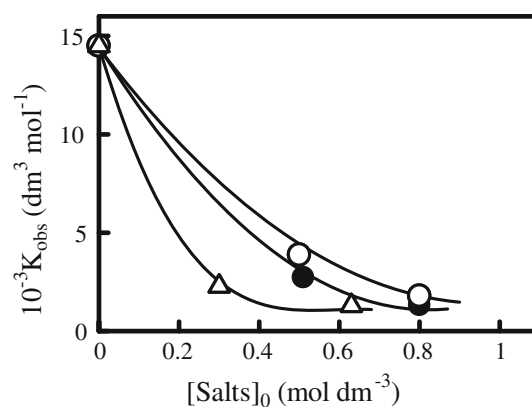
^a In a water–methanol mixture (phosphate buffer (0.01 mol dm⁻³))

dye solution. As shown in Fig. 2, a good linear relationship between $[\text{Calix-S8}]_0/\Delta E$ and $[\text{Calix-S8}]_0$ can be obtained. The association constants $K(\text{Th})$ for the 1:1 inclusion complex formation of Th in great excess of the three kinds of alkali salts (NaCl, KCl, and CsCl) can be determined from the slope and intercept according to Eq. 2. The results are listed in Table 1. The inclusion constants of Th with Calix-S8 decrease with increased concentrations of alkali salts. Calixarenes are important macrocyclic receptors. There are many reports concerning inclusion complexes with metal ions [16, 17]. The above results suggest a competitive binding aspect between the organic molecule Th and metal cations.

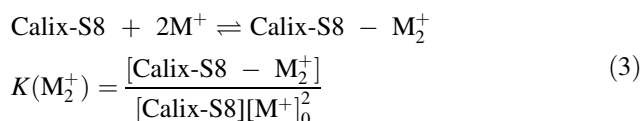
Effects of alkali salts on inclusion

Figure 3 shows changes in the inclusion constants of Th with Calix-S8 upon the addition of three kinds of alkali salts. Depending on the kind of alkali salt, the decrease in the inclusion constants is abrupt as the amount of alkali salts increases. The selectivity of metal cations in the inclusion of Calix-S8 decreases in the order of $\text{Cs}^+ < \text{Na}^+ < \text{K}^+$. Using the fluorescence-based method, Bakirci et al. [11, 18] suggested that inorganic cations compete with organic guests in the inclusion, and quantified the binding of inorganic cations with *p*-sulfonatocalix[4]arene. Therefore, in great excess of metal cations the apparent inclusion constant of the organic guest (Th) with Calix-S8 may fall below the actual inclusion constants.

Although the NMR-spectral proton shifts for the complexation of Calix-S8 with alkali metal cations could not be obtained in the Calix-S8 inclusion, the effects of the addition of alkali salts on the inclusion of Th suggest the binding of alkali metal cations with Calix-S8 in great excess of the alkali metal cations. Recent diffusion NMR measurements (DOSY) indicate that Calix-S8 forms 1:2

**Fig. 3** Dependence of alkali salts on the inclusion complexation of Th with Calix-S8 and cation-complexed Calix-S8 at 298 K. Filled circle NaCl, open triangle KCl, and open circle CsCl

host–guest complexes with Na^+ cations in excess sodium salt [12].



where $K(\text{M}_2^+)$ denotes the binding constant of metal cations with Calix-S8 and $[\text{M}^+]_0$ is the initial concentration of metal cations. On the basis of Bakirci's treatment ($[\text{cation}] \gg [\text{calixarene}]$) [11], the experimentally observed apparent inclusion constant (K_{obs}) of Th with Calix-S8 in great excess of metal cations can be expressed as follows:

$$K_{\text{obs}} \approx \frac{[\text{Calix-S8} - \text{Th}]}{[\text{Th}]([\text{Calix-S8}] + [\text{Calix-S8} - \text{M}_2^+])} \quad (4)$$

From Eqs. 1, 3, and 4, K_{obs} can be rewritten as:

$$K_{\text{obs}} \approx \frac{K(\text{Th})}{1 + K(\text{M}_2^+)[\text{M}^+]_0^2} \quad \text{for } [\text{M}^+]_0 \gg [\text{Calix-S8}]_0. \quad (5)$$

Equation 5 of the competitive binding model was applied to fit the data shown in Fig. 3. The $K(\text{M}_2^+)$ values of Calix-S8 were estimated to be 17, 60, and 11 dm⁶ mol⁻² for the Na^+ , K^+ , and Cs^+ cations, respectively. The binding constant for K^+ is large compared to those for Na^+ and Cs^+ . The stability for complexation with calixarenes might be determined by how well the guest fits into the host cavity, which is responsible for the size of the alkali cations complexed with Calix-S8.

In summary, an absorption-based method to quantify the binding of alkali metal cations with water-soluble Calix-S8 has been introduced based on competitive binding of the organic dye and metal cations. We have determined the binding constants of three kinds of alkali metal cations with Calix-S8 and have demonstrated the molecular recognition abilities of Calix-S8 for the size of metal cations.

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