Supporting Information

Inclusion Complexation and Self-association of Cucurbit[n]uril (n = 6, 7) and Diquat under Pseudo-physiological Conditions

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qNMR spectra were recorded over a spectral width of 12000 Hz using 8 scans without spinning. The pulse angle is 90°, and the repetition time is 64 s. DSS-\textit{d}_6 (FUJIFILM Wako Chemicals Co) is used as an internal reference in qNMR. CB[6] or CB[7] were dissolved in NaCl-D_2O. Purities of CB[6] or CB[7] were calculated using integrated values of the methyl proton signal of the trimethylsilyl group of DSS-\textit{d}_6 (Si-\textit{CH}_3) and the H^a and H^c proton signals of CB[6] or CB[7]. In the case of CB[7], although DSS-\textit{d}_6 forms the complex with CB[7], the integration value of Si-\textit{CH}_3 did not affect on the quantitative (-0.744 ppm). Purities of CB[6] or CB[7] were 82.9 % and 87.2 %, respectively.

Fig. S1  qNMR spectra of CB[6].

Fig. S2  qNMR spectra of CB[7].
The stoichiometry for the inclusion complexation of CB[7] and DQ was investigated via Job’s plot by using $^1$H NMR measurement when the sum of the CB[7] and DQ concentrations ([CB[7]] and [DQ]) was maintained at 1 and 10 mM; changes in the observed chemical shift ($\Delta \delta_{\text{obs}}$) of the DQ H$^4$ proton signal were used, and the stoichiometry was found from the plot of the $\Delta \delta_{\text{obs}}*[\text{DQ}]$ as function of the ratio $[\text{DQ}]/([\text{CB}[7]] + [\text{DQ}])$.

Fig. S3 Continuous variation plot (Job’s plot) by using $^1$H NMR measurement for the inclusion complexation of CB[7] and DQ: total concentration 1 mM in (a) pD1.2, (b) pD7.4, and (c) NaCl-D$_2$O; total concentration 10 mM in (d) pD1.2, (e) pD7.4, and (f) NaCl-D$_2$O.
The stoichiometry and association constant for the inclusion complexation between CB[7] and DQ were calculated via regression analysis at 30 - 65 °C in pD1.2, pD7.4, and NaCl-D$_2$O (Fig. S3, S5, and S7) while continuously increasing the CB[7] concentration ([CB[7]]) from 0 to 5 mM in a constant concentration of DQ ([DQ]; 0.5 mM). The following Eqn (1) was used for regression analysis. $K$ and $n$ are, respectively, the association constant and stoichiometry of CB[7] and DQ. $\Delta \delta_{\text{obs}}$ means ($\delta_{\text{DQ}} - \delta_{\text{obs}}$), $\Delta \delta_{\text{CX}}$ indicates ($\delta_{\text{DQ}} - \delta_{\text{CX}}$), and $\delta_{\text{DQ}}, \delta_{\text{CX}}$, and $\delta_{\text{obs}}$ are the chemical shifts (in ppm) of the H$_4$ protons of, correspondingly, DQ in a free state, the CB[7]-DQ inclusion complex, and the CB[7]-DQ mixture in the $^1$H NMR spectra. In Fig. S3, S5, and S7, $m_1, m_2$, and $m_3$ indicate that $\Delta \delta_{\text{CX}}$, $K$, and $n$, respectively. The thermodynamic parameters, that is, free energy change ($\Delta G$), enthalpy change ($\Delta H$), and entropy change ($\Delta S$), for the inclusion complexation of CB[7] and DQ were derived from Van’t Hoff plots by using association constants.

$$n \text{ CB[7]} + \text{ DQ} \xrightleftharpoons[K]{\text{CB[7]-DQ}}$$

$$[\text{CB[7]}] = \left[ \frac{1}{\left( \frac{\Delta \delta_{\text{CX}}}{\Delta \delta_{\text{obs}}} - 1 \right) K} \right]^{1/n} + n \frac{\Delta \delta_{\text{obs}}}{\Delta \delta_{\text{CX}}} [\text{DQ}] \quad \cdots \text{Eqn (1)}$$
**Fig. S4** Regression analysis by using Eqn (1) based on $\Delta \delta_{\text{obs}}$ of the H$_4$ proton signal of DQ (0.5 mM DQ) at 30 - 65 °C in pD1.2: (a) 30 °C, (b) 35 °C, (c) 40 °C, (d) 45 °C, (e) 50 °C, (f) 55 °C, (g) 60 °C, and (h) 65 °C.

**Fig. S5** Van’t Hoff plots for the inclusion complexation between CB[7] and DQ in pD1.2.

Van’t Hoff plots for the inclusion complexation between CB[7] and DQ (0.5 mM DQ) in pD1.2.
Fig. S6 Regression analysis by using Eqn (1) based on Δδ_{obs} of the H^4 proton signal of DQ (0.5 mM DQ) at 30 - 65 °C in pD7.4: (a) 30 °C, (b) 35 °C, (c) 40 °C, (d) 45 °C, (e) 50 °C, (f) 55 °C, (g) 60 °C, and (h) 65 °C.

Fig. S7 Van’t Hoff plots for the inclusion complexation between CB[7] and DQ in pD7.4.
Fig. S8 Regression analysis by using Eqn (1) based on Δδ_{obs} of the H^4 proton signal of DQ (0.5 mM DQ) at 30 – 65 °C in NaCl-D_2O: (a) 30 °C, (b) 35 °C, (c) 40 °C, (d) 45 °C, (e) 50 °C, (f) 55 °C, (g) 60 °C, and (h) 65 °C.

Van't Hoff plots for the inclusion complexation between CB[7] and DQ in NaCl-D_2O

Fig. S9 Van’t Hoff plots for the inclusion complexation between CB[7] and DQ (0.5 mM DQ) in NaCl-D_2O.
The association constant for the inclusion complexation of CB[7] and DQ was calculated via Benesi-Hildebrand method in pD1.2, pD7.4, and NaCl-D_2O while continuously increasing the CB[7] concentration ([CB[7]]) from 0 to 5 mM in a constant concentration of DQ ([DQ]; 0.05 mM). The UV spectroscopy was conducted by using a UVmini-1240 (SHIMADZU, Japan); the absorbance was recorded at 330 nm and 35 °C. The following Eqn (2) was used for regression analysis. \( K \) is the association constant of CB[7] and DQ, \( \Delta E \) means \( (A_{\text{obs}} - A_{\text{DQ}}) \), \( \Delta \varepsilon \) indicates \( (\varepsilon_{\text{complex}} - \varepsilon_{\text{DQ}}) \), and \( A_{\text{obs}} \) and \( A_{\text{DQ}} \), \( \varepsilon_{\text{complex}} \) and \( \varepsilon_{\text{DQ}} \) are the absorbance of CB[7]-DQ mixture and DQ in a free state, and the molar absorption coefficient of the CB[7]-DQ inclusion complex and DQ in a free state.

\[
\text{CB[7]} + \text{DQ} \rightleftharpoons \text{CB[7]}-\text{DQ}
\]

\[
\frac{1}{\Delta E} = \frac{1}{K \Delta \varepsilon [\text{DQ}][\text{CB[7]}]} + \frac{1}{\Delta \varepsilon [\text{DQ}]} \quad \cdots \cdots \text{Eqn (2)}
\]
Fig. S10 Regression analysis by using Eqn (2) based on $\Delta E$ at 35 °C in (a) pD1.2, (b) pD7.4, and (c) NaCl-D$_2$O.
The \( n \) and \( K \) for the complexation between CB[7] and DQ were calculated via regression analysis by using Eqn (1) at 30 - 65 °C in NaCl-D\(_2\)O. The CB[7] concentration continuously was increased from 0 to 10 mM in a constant concentration (1 mM DQ). The \( \Delta G, \Delta H, \) and \( \Delta S \) for the inclusion complexation of CB[7] and DQ were derived from Van’t Hoff plots by using association constants. The association constant at 35 °C was 484 M\(^{-1}\), and \( \Delta G, \Delta H, \) and \( T\Delta S \) were \(-15.8 \text{ kJ/mol}, \ -1.1 \text{ kJ/mol} \) and \(-14.7 \text{ kJ/mol} \), respectively.
Fig. S11 Regression analysis by using Eqn (1) based on $\Delta \delta_{\text{obs}}$ of the $H^4$ proton signal of DQ (1 mM DQ) at 30 - 65 °C in NaCl-D$_2$O: (a) 30 °C, (b) 35 °C, (c) 40 °C, (d) 45 °C, (e) 50 °C, (f) 55 °C, (g) 60 °C, and (h) 65 °C.
Van’t Hoff plots for the inclusion complexation between CB[7] and DQ in NaCl-D₂O.

Fig. S12 Van’t Hoff plots for the inclusion complexation between CB[7] and DQ (1 mM DQ) in NaCl-D₂O.
Fig. S13 Behavior of CB[7] or DQ, as revealed via $^1$H NMR when increasing the CB[7] or DQ concentrations in pD1.2, pD7.4, and NaCl-D$_2$O: CB[7] in (a) pD1.2, (b) pD7.4, and (c) NaCl-D$_2$O; DQ in (d) pD1.2, (e) pD7.4, and (f) NaCl-D$_2$O. $H^a$, $H^b$, and $H^c$ indicate the proton signals of CB[7]. $H^3$, $H^4$, $H^5$, and $H^6$ indicate the proton signals of DQ.
Fig. S14 Rotating-frame Overhauser effect (ROE) observed between CB[7] and DQ when forming the inclusion complex in pH7.4: (a) $^1$H NMR spectrum of CB[7] and DQ; (b) one-dimensional (1D) ROESY spectrum irradiated to the $H^a$ proton of CB[7]; (c) 1D ROESY spectrum irradiated to the $H^7$ proton of DQ. The half-arrows in 1D ROESY spectra indicate positions of the irradiated proton signals.

Fig. S15 ROE observed between CB[7] and DQ when forming the inclusion complex in NaCl-D$_2$O: (a) $^1$H NMR spectrum of CB[7] and DQ; (b) 1D ROESY spectrum irradiated to the $H^a$ proton of CB[7]; (c) 1D ROESY spectrum irradiated to the $H^7$ proton of DQ. The half-arrows in 1D ROESY spectra indicate positions of the irradiated proton signals.