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Host–Guest Chemistry

Supramolecular Recognition of Amino Acids by Twisted Cucurbit[14]uril

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Abstract: Binding interactions between twisted cucurbit[14]uril (*t*Q[14]) and twenty standard amino acids (AAs) have been investigated by NMR spectroscopy and isothermal titration calorimetry (ITC) in aqueous HCl solutions and in DMSO. The results showed that *t*Q[14] displays clear binding affinity for AAs with a positively charged side chain or containing an aromatic ring, but weaker binding affinity for AAs with hydrophobic or polar side chains, with the binding mode depending on the type of side chain present in the AAs.

The study of amino acid host–guest recognition is of considerable interest in relation to chiral recognition, molecular sensing, detection and possibly also to the development of amino acid (AA) separation technologies.[1–3] Cucurbit[*n*]urils (Q[*n*]s) are synthetic macrocyclic host molecules, generally possessing a rigid hydrophobic cavity and two identical carbonyl-fringed portals, and have been widely used in host–guest chemistry.[4] Amino acid, peptide sequence-specific and protein recognition by the Q[*n*]s with $n=6,7,8$ has been extensively studied.[5–7] Among the Q[*n*] homologues, Q[6] binds to some AAs bearing hydrophobic side chains, such as Phe, Ala, and Val[8,9] while Q[7] shows higher binding affinity towards Phe in aqueous solution compared to other AAs such as Lys, Arg, His, Tyr, and Trp with different side chains.[10–12] The host-guest binding affinity of Q[*n*]s towards AAs has been rationalized in terms of a combination of electrostatic interactions involving their carbonyl portals and hydrophobic interactions involving their cavities.[13,14] The larger cavity of Q[8] can simultaneously and selectively bind methyl viologen and secondary aromatic AAs. Complex formation in this case is driven by hydrophobic interactions as well as charge transfer between the guests and the cavity of Q[8].[15,16] Furthermore, the use of supramolecular Q[*n*] host–guest chemistry has been employed for such applications as the real-time monitoring of enzymatic reactions and the fluorimetric detection and discrimination of AAs.[17–19]

Recently, a higher Q[*n*] homologue, *t*Q[14], that could further broaden the potential applications of cucurbit[*n*]urils in supramolecular chemistry, was discovered by our group. Indeed, *t*Q[14] is the largest cucurbit[*n*]uril yet characterized, with 14 normal glycoluril units linked by 28 methylene bridges and showing a 180° twist. It has two kinds of cavities (a central cavity and two side cavities) and adopts a folded, figure-of-eight conformation. As a result, it does not have a "normal" circular cavity like the lower cucurbit[*n*]urils ($n=5$, 6, 7, 8, 10) and shows structural flexibility; it is soluble in water and DMSO.[20] *t*Q[14] also shows unusual binding behavior with various guest molecules in aqueous solution. For example, 4,4′-bipyridyl derivatives are capable of threading the *t*Q[14] cavity to form novel shell-like inclusion complexes.[21] Moreover, *t*Q[14] can accommodate one or two alkyldiammonium cations due to its two different kinds of cavities. This feature is rarely observed in conventional supramolecular hosts.[22] In the present work, we have investigated the binding interactions between *t*Q[14] and the 20 standard AAs in aqueous HCl solution by isothermal titration calorimetry (ITC) and in DCl/D_2O and [D6]DMSO by ^{1}H NMR spectroscopy.[23] The results show that *t*Q[14] displays stronger binding affinity towards AAs with a positively charged side chain or ones incorporating an aromatic ring, and weaker binding affinity towards AAs with an uncharged or negatively charged side chain. Moreover, as expected, the binding mode is seen to depend on the nature of the side chains of the AAs

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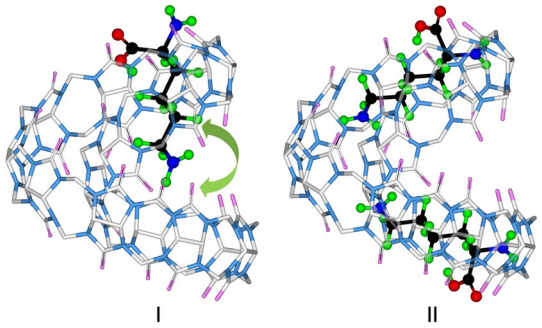
and the solvent (D_2O or [D6]DMSO) employed. Scheme 1 shows possible binding modes of *t*Q[14] with Lys.



Scheme 1. The possible binding modes of *t*Q[14] with Lys.

Isothermal titration calorimetry (ITC) was employed to investigate the binding interactions of *t*Q[14] with 20 L-amino acids in aqueous HCl (0.01 M). Equilibrium association constants (K_a) and thermodynamic parameters from the ITC experiments are listed in Table 1, and ITC profiles are shown in Figure S1, Supporting Information. It can be seen that Lys, Arg, and His showed the highest binding affinities (10^5–10^6 M^{-1}) toward *t*Q[14] among the AAs investigated. The binding affinities of the AAs with aromatic side chains (Phe, Tyr, and Trp), as well as Met and Cys with sulfhydryl groups (10^4–10^5 M^{-1}), are higher than those of the other AAs (ITC experiments showed no effective interaction of *t*Q[14] with these AAs, see Figure S1, SI). The results are in accord with *t*Q[14] showing high affinity not only for AAs with positively charged side chains (Lys, Arg, His, Met, and Cys) but also for AAs with aromatic side chains (Phe, Trp, and Tyr). Conversely, *t*Q[14] shows lower affinities for AAs with hydrophobic uncharged side chains or polar side chains. The observations may be explained in terms of a combination of ion–dipole and electrostatic interactions between the positively charged side chains of the AAs and the polar carbonyl groups of *t*Q[14] and hydrophobic interactions between the aromatic moieties of the AAs and the macrocyclic cavity, respectively.

Table 1. Stability constants K_a (M^{-1}) and thermodynamic parameters ΔH and $T\Delta S$ (kJ mol^{-1}) for complex formation of amino acids with twisted cucurbit[14]uril in aqueous HCl solution (0.01 M) at 25 °C.

AA	K_a [M^{-1}]	ΔH [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]
Lys[a]	$(6.29\pm1.07)\times10^6$	-44.92 ± 2.43	-6.11 ± 0.79
Arg[a]	$(3.64\pm0.58)\times10^5$	-45.71 ± 0.74	-13.97 ± 1.35
His[b]	$(2.05\pm0.62)\times10^5$	-59.89 ± 8.16	-29.56 ± 2.55
Phe[b]	$(1.69\pm0.48)\times10^5$	-8.03 ± 0.49	22.60 ± 3.77
Tyr[b]	$(2.23\pm0.47)\times10^4$	-5.64 ± 0.73	19.38 ± 1.92
Trp[b]	$(1.53\pm0.38)\times10^4$	-7.73 ± 0.89	17.83 ± 1.38
Met[b]	$(2.37\pm0.12)\times10^4$	-6.63 ± 0.58	20.34 ± 2.02
Cys[b]	$(1.48\pm0.21)\times10^4$	-7.62 ± 0.77	16.95 ± 3.73

[a] Concentration of *t*Q[14] in the sample cell (1.3 mL): 5×10^{-5} mol·L^{-1}. Each ITC titration involved addition of the AA solution (2×10^{-3} mol·L^{-1}, 5 μL aliquots) to the *t*Q[14] solution. [b] Concentration of *t*Q[14] in the sample cell (1.3 mL): 1×10^{-4} mol·L^{-1}. Each ITC titration involved addition of the AA solution (2×10^{-3} mol·L^{-1}, 10 μL aliquots) to the *t*Q[14] solution.

The thermodynamic parameters for *t*Q[14]/AAs binding listed in Table 1 indicate that the binding of *t*Q[14] with Arg, Lys, and His is essentially an enthalpy-driven process ($|\Delta H| > |T\Delta S|$). The main contributions to the reaction enthalpies being from ion–dipole interaction between the protonated amino group of each AA and the carbonyl groups of *t*Q[14] as well as hydrophobic interaction between the side-chain moiety of each AA and the cavity of the macrocycle. For the other five AAs, complexation is an entropically controlled process ($|T\Delta S| > |\Delta H|$) which is in keeping with the presence of hydrophobic interaction arising from the inclusion of the side chain moieties of the AAs inside the cavity of *t*Q[14].

^{1}H NMR spectroscopy was utilized to probe the host–guest interactions of *t*Q[14] with AAs. Notably, Lys and Arg showed generally greater spectral changes at pD 2 (Figure 1 and Figure S2, SI). Titration ^{1}H NMR spectra obtained using a fixed amount of *t*Q[14] and various equivalents of Lys are shown in Figure 1. The side-chain proton signals for Lys showed an upfield shift of about 0.3 ppm, and the signal for the α proton showed a downfield shift of about 0.2 ppm (Figure 1b to 1f). This may reflect that the α proton lies outside the portion of the side chain of Lys included in the cavity. It should be noted that the proton signals are averaged signals of the free and bound Lys which are in rapid exchange on the NMR time scale. Titration ^{1}H NMR spectra at pD 2 revealed that the interaction of *t*Q[14] with Arg is similar to that obtained with Lys (Figure S2, SI). The ^{1}H NMR results are in accord with the respective side-chain moieties of Lys and Arg being subject to shielding by the host through being included in the cavity of *t*Q[14] in solution. However, the titration ^{1}H NMR spectra obtained employing DCl do not imply that there are two different interaction modes as illustrated in Scheme 1.

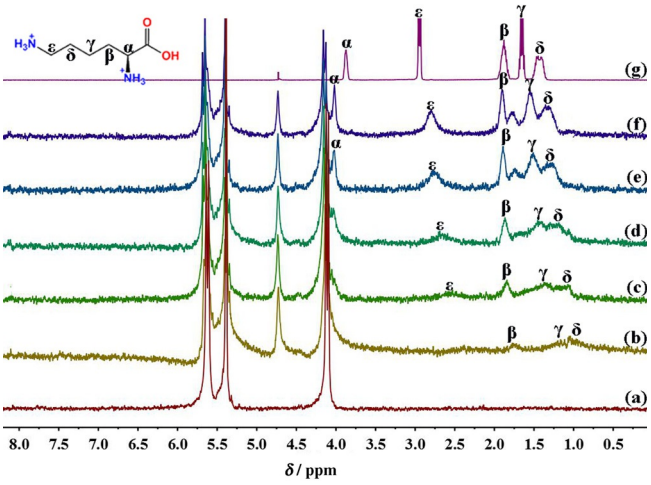


Figure 1. ^{1}H NMR spectra (500 MHz, D_2O) of *t*Q[14] in the absence (a) and presence of 0.05 (b), 0.12 (c), 0.54 (d), 1.20 (e), and 4.00 (f) equiv of Lys at pD 2 at 25 °C. (g) ^{1}H NMR spectrum of Lys at pD 2 at 25 °C.

In a recent study concerned with the binding interactions between *t*Q[14] and a series of alkyldiammonium ions in both aqueous solution and [D6]DMSO, we observed a similar situation with the binding of HCl salts of butanediamine or hexanediamine; only the titration ^{1}H NMR spectra in [D6]DMSO could be used to distinguish two different host–guest interaction modes.[22] Thus, for comparison, the titration ^{1}H NMR spectra of *t*Q[14] with Lys in [D6]DMSO were acquired (Figure 2). The results showed different binding behavior for *t*Q[14] in [D6]DMSO as the number of equivalents of Lys were increased (Figure 2b to 2f): only one set of amino proton signals (A′, B′) and the side-chain proton signals of Lys (β′, γ′, δ′) were observed upon the addition of a small amount of Lys (Figure 2b and 2c), suggesting that the *t*Q[14] host shields both the amino groups and side chain of Lys in [D6]DMSO. Based on our previous study,[22] each *t*Q[14] molecule could encapsulate a Lys molecule in its central cavity (mode I in Scheme 1). However, a second set of Lys signals (based on amino protons A″, B″) appeared upon the addition of increasing numbers of equivalents of Lys (Figure 2d–2f), suggesting adoption of the second binding mode by the *t*Q[14]/Lys system. Although the detailed interaction mode cannot be discerned from the available data, we presume that two Lys molecules may be simultaneously encapsulated in two side cavities of *t*Q[14] (mode II in Scheme 1).[22] Thus, the binding modes could be very similar to those for *t*Q[14] binding with protonated butanediamine or hexanediamine as discussed previously.[22] It is noted that ITC profiles in neutral aqueous solution also implied two binding modes for interactions of *t*Q[14] with two Lys or two Arg (see Figure S3, and Table S1, SI). Moreover, titration ^{1}H NMR spectra in [D6]DMSO also indicated two different binding interactions for *t*Q[14] with Arg, similar to those seen with Lys (Figure S4, SI).

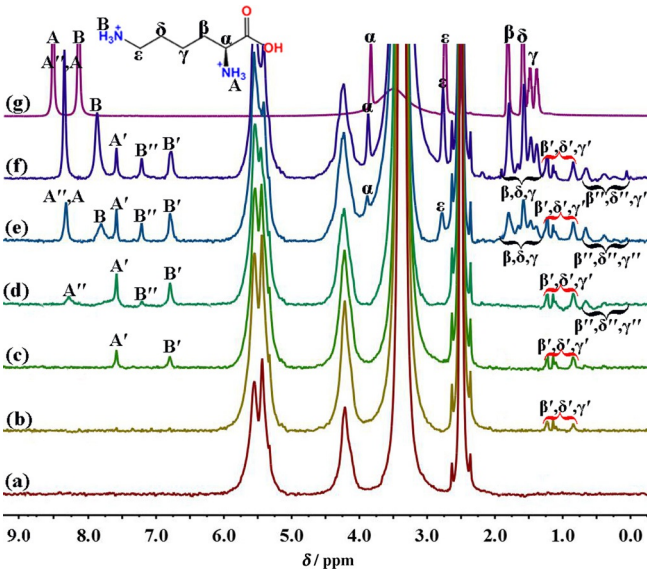


Figure 2. ^{1}H NMR spectra (500 MHz, 0.50 mL [D6]DMSO) of *t*Q[14] (5 mm) in the absence (a) and presence of 0.20 (b), 0.80 (c), 1.20 (d), 2.50 (e), and 5.00 (f) equiv of Lys. (g) ^{1}H NMR spectrum of Lys in 0.50 mL [D6]DMSO at 25 °C.

The imidazole proton signals of His bound to *t*Q[14] show upfield shifts, in accord with this ring being encapsulated in *t*Q[14] at pD 2 (Figure 3). The imidazole nitrogen of His is expected to be protonated at pD 2, which is expected to promote tighter binding with *t*Q[14] through hydrogen bonding and ion–dipole interaction of the protonated amine group with the portal carbonyl oxygen atoms of the macrocycle—in addition to the aforementioned cavity interaction. This is reflected by the observed more favorable enthalpy and a less favorable entropy of binding in this case. All of the experimental results imply the presence of only one host–guest interaction mode for the *t*Q[14]/His system. This is also the case for the binding of *t*Q[14] with AAs bearing an aromatic moiety (Phe, Trp, and Tyr); the aromatic ring proton signals of these AAs are subject to upshift shifts upon binding to *t*Q[14] (Figure S5, SI).

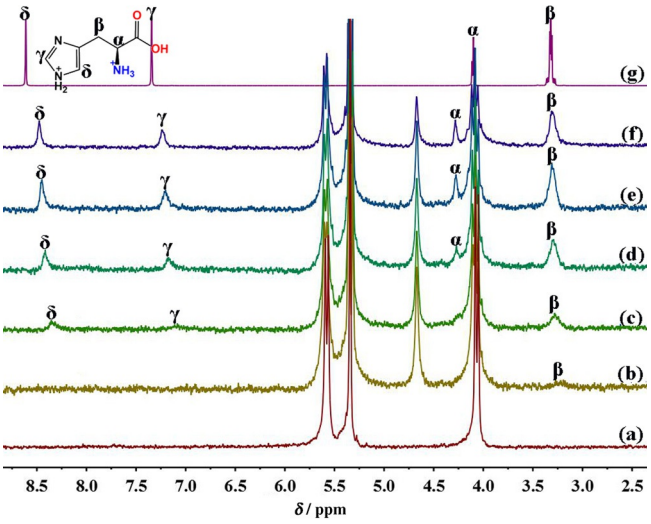


Figure 3. ^{1}H NMR spectra (500 MHz, D_2O) of *t*Q[14] in the absence (a) and presence of 0.20 (b), 0.25 (c), 0.60 (d), 1.25 (e), and 2.00 (f) equiv of His at pD 2 at 25 °C. (g) ^{1}H NMR spectrum of His at pD 2 at 25 °C.

Based on the above titration ^{1}H NMR experiments, the interactions of *t*Q[14] with the remaining AAs can be subdivided into two groups. For the first group, the side-chain proton signals show a clear upfield shift, suggesting that the side chains preferentially reside in the cavity of *t*Q[14]. The particular AAs showing this behavior are Cys, Met, Pro, Gly (Figure S6, SI). In the second group, the side-chain proton signals show almost no change or are even downfield shifted, suggesting that the respective side chains are largely excluded from the cavity of *t*Q[14]. The AAs showing this behavior are Leu, Ile, Ala, Val, Ser, Thr, Gln, Glu, Asn, and Asp (Figure S7, SI). Clearly affinity of the side chain of individual AAs for the portal oxygens as well as for the cavity of *t*Q[14] play key roles in the formation of stable host–guest inclusion complexes.

The proton resonances of AAs with hydrophobic side chains generally show upfield shifts. For example, spectra pertaining to the interaction of *t*Q[14] with Cys (as a representative of the first group) are shown in Figure 4. The β proton resonance of Cys is subject to a ca. 0.4 ppm upfield shift during the titration

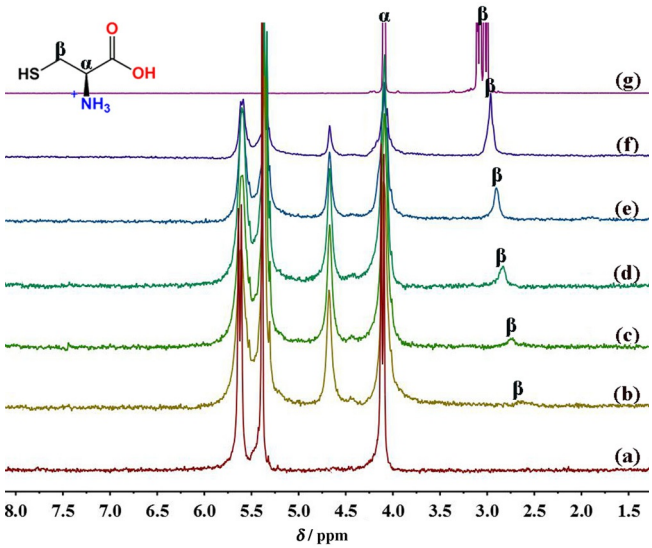


Figure 4. ^{1}H NMR spectra (500 MHz, D_2O) of *t*Q[14] in the absence (a) and presence of 0.15 (b), 0.2 (c), 0.4 (d), 2.5 (e), and 10 (f) equiv of Cys at pD 2 at 25 °C. (g) ^{1}H NMR spectrum of Cys at pD 2 at 25 °C.

process. In contrast, the signals for Glu (as a representative of the second group) show almost no change on addition of various amounts of *t*Q[14]; the α proton resonance is even shifted slightly downfield shift (Figure 5). The side chains in such AAs contain a carbonyl or acylamino moiety, such that repulsion will occur between the side chain and the portal carbonyl groups of *t*Q[14]. Unlike the situation for Lys or Arg, ^{1}H NMR titration data for *t*Q[14] in [D6]DMSO with the other AAs gave no evidence for significant interaction. That is, only very minor changes of the ^{1}H chemical shifts were observed on stepwise addition of several equivalents of these AAs (Figures S8–S10, SI) to *t*Q[14] in [D6]DMSO.

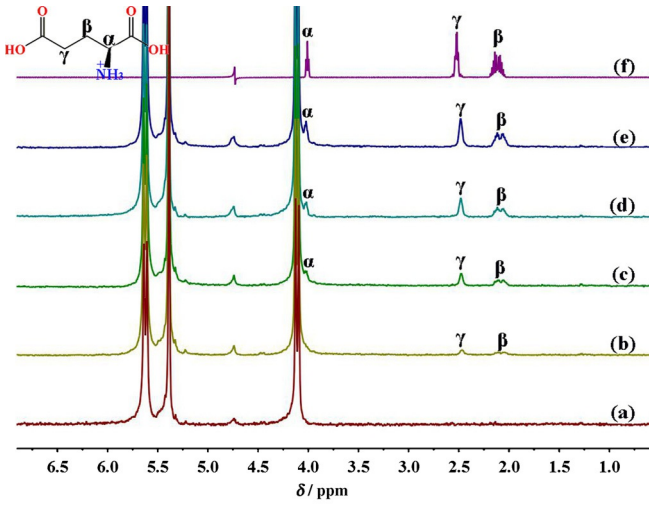


Figure 5. ^{1}H NMR spectra (500 MHz, D_2O) of *t*Q[14] in the absence (a) and presence of 0.25 (b), 0.60 (c), 0.80 (d), and 1.40 (e) equiv of Glu at pD 2 at 25 °C. (f) ^{1}H NMR spectrum of Glu at pD 2 at 25 °C.

In summary, we have investigated the binding interactions of 20 AAs with *t*Q[14] in both aqueous acid solution and DMSO by ^{1}H NMR spectroscopy as well as by isothermal titration calorimetry in aqueous HCl. Comparing the binding affinities, Lys and Arg were observed to form more stable complexes than the aromatic AAs. *t*Q[14] was also shown to interact with the remaining AAs displaying a variety of different side chains. Overall, in aqueous solution the different binding affinities of *t*Q[14] towards the 20 standard AAs may be rationalized in terms of a combination of electrostatic interactions at the carbonyl portals and cavity hydrophobic interactions with the side chains of the respective AAs. In DMSO, however, *t*Q[14] only interacts strongly with Lys and Arg, which contain positively positively charged side chains, while weaker binding occurs with the remaining 18 AAs. Thus, it might be inferred that ion–dipole interactions are a dominant driving force for host–guest complexation by these systems.

Experimental Section

Materials

20 L-forms of the AAs were obtained from Aladdin Industrial Corporation (Shanghai, China) and were used without further purification. *t*Q[14] was prepared and purified according to previously published methods.[20]

Characterization

All ^{1}H NMR spectra, including those for titration experiments, were recorded at 25 °C on a Varian Inova-500 spectrometer. D_2O was used as a field-frequency lock and the observed chemical shifts are reported in parts per million (ppm) relative to that for the internal standard (TMS at 0.0 ppm).

All calorimetric titrations are performed using a Nano ITC instrument (TA, USA) employing aqueous HCl solutions (0.01 M). The heat evolved was recorded at 298.15 K. The heat of dilution was corrected by injecting the guest solution (free guest) into the aqueous HCl solution and subtracting the values from the corresponding values obtained for the host-guest titration. Computer simulations (curve fitting) were performed using the Nano ITC analytical software. For Arg and Lys, the concentration of *t*Q[14] in the sample cell (1.3 mL) was 5×10^{-5} mol L^{-1}. A typical ITC titration was carried out by titrating the AAs solution (2×10^{-3} mol/L, 5 μL aliquots, at 250-second intervals) into a *t*Q[14] solution. For the remaining 18 AAs(containing His, Phe, Tyr, Trp, Met, Cys, Pro,Leu, Ile, Gly, Ala, Val, Ser, Thr, Gln, Glu, Asn, or Asp), the concentration of *t*Q[14] in the sample cell (1.3 mL) was 1×10^{-4} mol L^{-1}. The ITC titrations were carried out by titrating AAs solution (2×10^{-3} mol L^{-1}, 10 μL aliquots, at 250-second intervals) into the *t*Q[14] solution.

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Keywords: amino acids · host–guest chemistry · side chains · supramolecular recognition · twisted cucurbit[14]uril

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