Dynamics of Molecular Recognition Involving Cucurbituril[1]

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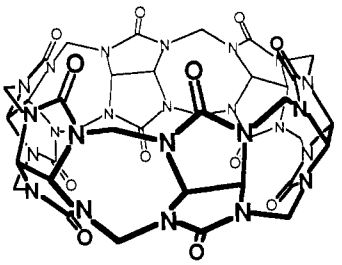
Abstract: For the synthetic receptor cucurbituril, the rate of inclusion complex formation correlates with the molecular diameter of alkylammonium ion ligands but not with the thermodynamic stability of the complexes formed. Measurements of ^{13}C NMR spin-lattice relaxation allow comparison of molecular tumbling motions of the receptor with those of bound ligands, by determination at their respective correlation times. Guest ions appear to rotate relatively freely within cucurbituril, irrespective of the stability of the complexes. Results are interpreted in terms of shape complementarity between receptor and ligand.

Understanding the phenomenon of biochemical specificity, as observed in enzyme–substrate, antigen–antibody, or hormone–receptor interactions, etc., represents a challenge that is yielding to contemporary investigations. The recent availability of synthetic macrocyclic receptors has greatly aided progress in the area of molecular recognition, by providing models in which systematic study of noncovalent bonding may be pursued. For the most part, these have been *thermodynamic* investigations, in which the factors contributing to the stability of molecular complexes have been explored. While fruitful, such an approach only partly addresses the question of biochemical specificity. Biological processes are inherently *dynamic* in nature. For example, it is recognized that enzymes in vivo commonly operate with substrate concentrations below K_m (i.e., with less than "saturating" amounts of reactants in the steady state). Furthermore, conversion of bound substrate may be more rapid than reversal of the binding step. This means that enzymic specificity (in the sense of selection between competing potential substrates) may be a *kinetic* phenomenon as much as it is a *thermodynamic* one. Clearly, the dynamics of molecular recognition deserve more attention.

Cucurbituril (**1**) is a synthetic molecular receptor that is readily assembled from urea, glyoxal, and formaldehyde [$2H_2NCONH_2 + CHOCHO \rightarrow C_4H_6N_4O_2$ (glycoluril), $6C_4H_6N_4O_2 + 12CH_2O \rightarrow C_{24}H_{36}N_{24}O_{12}$ (cucurbituril)].[2] It has a hollow interior, which is connected to the exterior by two carbonyl-fringed portals. Studies of the equilibrium binding of aliphatic and aromatic ammonium ions within **1** have previously been reported,[3,4] as has



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catalysis by **1** of a specific cycloaddition.[5] Typical ligands of high affinity for **1** are *n*-alkyl-, and cycloalkylammonium ions of 4–6 carbon atoms. Cross-sectional representations of the *n*-pentyl- and cyclopentanemethylammonium ion complexes of **1** are presented in Figure 1. The noncovalent binding interactions may be conceptually divided into two contributions: an ion–dipole attraction leading to coordination of the cationic ammonium moiety to the collected carbonyl oxygens that surround each portal,[3] and a hydrophobic effect associated with encapsulation of hydrocarbon portions of the ligand.[4]

This article focuses upon certain aspects of the *dynamics* of molecular recognition with **1**. The kinetics of ligation as a function of substrate size are investigated, and motions of the ligand within the complexes of **1** are examined.

Table I. Ligand–Receptor Kinetic Data for **1**

ligand[a]	10^5K_d, M	10^5k_{out}, s^{-1}	k_{in},[b] s^{-1} M^{-1}	T_c, °C
1. $(CH_3)_2CH(CH_2)_2NH_3^+$	2.8	37 (±3)	13.3	
2. $(CH_3)_2CH(CH_2)_3NH_3^+$	24	120 (±3)	5.0	
3. 2-$C_4H_3SCH_2NH_3^+$[c]	0.43	9.3 (±0.3)	21.4	
4. c-$(CH_2S)_2CHCH_2NH_3^+$[d]	0.17	1.6 (±0.1)	9.2	
5. c-$(CH_2)_4CHCH_2NH_3^+$	0.30	1.6 (±0.1)	5.5	
6. c-$((CH_2)_3CHCH_2NH_3^+$	0.27	1600 (±300)	5900	
7. c-$(CH_2)_2CHCH_2NH_3^+$	6.8	$>10^7$	$\geq 10^6$	<40
8. $CH_3(CH_2)_3NH_3^+$	1.0			74 (±3)
9. $CH_3(CH_2)_4NH_3^+$	4.2			89 (±3)
10. $CH_3(CH_2)_5NH_3^+$	44			89 (±3)
11. $CH_3(CH_2)_6NH_3^+$	990			45 (±5)

[a] In solution 1:1 (v/v) HCO_2H–D_2O. [b] $k_{in} = k_{out}/K_d$. [c] 2-Thiophenemethylammonium ion. [d] 2-Dithiolanemethylammonium ion.

Results

The chief technique for studying ligand–receptor interactions involving cucurbituril is NMR spectroscopy. Substrates bound within **1** typically exhibit ^1H NMR signals 1 ppm in the direction of higher field relative to the corresponding resonances of the same species free in solution. This permits determination of dissociation constants by the simple expedient of allowing two potential ligands to compete for a limited amount of **1**.[3a] Integration of the appropriate NMR signals after attainment of equilibrium permits computation of the relative affinity of the two ligands for **1**. The absolute dissociation constant for a reference substrate is known (*p*-$CH_3C_6H_4CH_2NH_3^+$, $K_d = 3.1 \times 10^{-3}$ M). By comparison with this ligand, a large number of dissociation constants for cucurbituril have been determined.[3] Values of K_d for a selected group of substrates that will be given kinetic consideration are recorded in Table I (note: smaller magnitude for K_d = tighter binding).

In the course of collecting dissociation constant data, it was noted that in a number of instances equilibrium was only slowly attained when a second ligand was added to a pre-existing solution of **1** complexed with an initial ligand. This makes feasible a systematic investigation of the kinetics of ligation. It was shown that the rate of ligand substitution in such cases is independent of the structure or the concentration of the displacing ligand; i.e., the first ligand spontaneously *dissociates* (or is replaced by solvent), and then in a subsequent rapid step the new complex is formed.[3b] For the initial six substrates listed in Table I the rate constant for dissociation was determined in this fashion by exponential curve fitting of NMR integrals vs time, yielding k_{out}. Knowledge of K_d and k_{out} allows calculation of a rate constant for association, k_{in}, also listed in Table I. It may be observed that considerable variation exists in rate and equilibrium parameters according to the structure of the coordinating alkylammonium

(1) Taken in part from the Ph.D. Thesis of N.-Y. Shih, University of Illinois at Chicago, 1981: *Diss. Abstr. Int. B* **1982**, *42*, 4071.
(2) Freeman, W. A.; Mock, W.; L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1981**, *103*, 7367.
(3) (a) Mock, W. L.; Shih, N.-Y. *J. Org. Chem.* **1986**, *51*, 4440. (b) This technique has previously been used: Anthonsen, T.; Cram, D. J. *J. Chem. Soc., Chem. Commun.* **1983**, 1414.
(4) Mock, W. L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1988**, *110*, 4706.
(5) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Manimaran, T. L. *J. Org. Chem.* **1983**, *48*, 3619.

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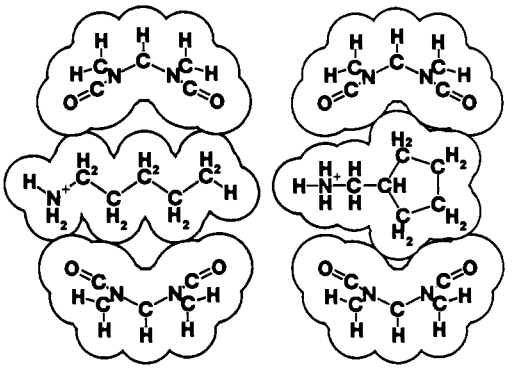

Figure 1. Conjectured cross-sectional representations of ligand–receptor complexes for **1**·$CH_3(CH_2)_4NH_3^+$ and **1**·c-$(CH_2)_4CHCH_2NH_3^+$. Outlines drawn to van der Waals radii (maximum projection for all atoms upon axial rotation of **1**, crystallographically determined interatomic distances for **1**).

ion. The most pertinent observation is that methyl branching (or presence of a five-membered ring) in the alkyl portion of the substrate uniformly retards insertion of a ligand into cucurbituril, relative to the cases of less bulky analogues (cf., k_{in}).

Successful measurement of rate constants with the foregoing ligands is contingent upon an observation of separate signals for bound and free (uncomplexed) substrates. For certain ligands the exchange between the interior of **1** and the uncomplexed state is *not* always slow on the NMR time scale. For these substrates coalescense behavior is noted for the NMR signals as the temperature is varied for a solution containing a 2:1 mole ratio of ligand to receptor. Separate signals, which are observed for the two ligand environments at low temperature, are seen to merge as the sample is heated. For the *n*-alkylammonium entries in the lower portion of Table I an approximate coalescence temperature is recorded (T_c). The seventh entry, cyclopropanemethylammonium ion, represents a transitional example. Its dissociation from **1** is too rapid for measurement by the displacement technique, yet coalescense must occur at a lower temperature than 40 °C. On the basis of the latter observation a limiting value for k_{out} may be estimated from the difference in chemical shift for free and complexed ligands and the upper limit on coalescense temperature. The *n*-alkylammonium ions listed in Table I (entries 8–11) apparently extrude from **1** with comparable ease; coalescense temperatures are similar in spite of differing molecular length.

There is a second dynamic aspect of ligand–receptor complexation that we wish to consider briefly. The *molecular motions* of guests ensconced within cucurbituril are of interest. Information on the relative tumbling rates in solution of receptor and ligand is accessible from NMR spectroscopy, as has been demonstrated for cyclodextrin[6] and cryptate[7] complexes. The predominant relaxation process for ^{13}C nuclei in CH_2 and CH groups arises from dipolar interactions with directly attached hydrogen nuclei. For a ^{13}C nucleus the measured spin–lattice relaxation time T_1 yields a correlation time τ_c for its motions according to the equation $\tau_c = (2.8 \times 10^{-11})r^6/nT_1$, in which n is the number of hydrogens attached to a particular carbon nucleus at a distance r. Suitable carbon atoms are present in both cucurbituril and many of its ligands. Hence, NMR relaxation rate measurements on the complexes allow an examination of whether stability of a complex (as measured by K_d value) is reflected in the relative freedom of guest to move about within the host.

Table II contains pertinent data for a series of selected cucurbituril–ligand complexes in aqueous formic acid. Each complex exhibits slow exchange, and each was examined as a stoichiometric adduct. Spin–lattice relaxation (T_1) measurements were carried out by the standard inversion–recovery technique. In general, satisfactory decay constants were obtained for the FT–NMR signals from several carbon positions in each of the ligands, as well as for the two types of $^{13}C(H)$ atoms in **1**. These were converted into τ_c values by the appropriate equation given previously. Typically, τ_c values for the receptor fall in the range of 200–300 ps (a reasonable value for a molecule of its size), with those of the bound ligand nearly an order of magnitude lower. The τ_c values for uncomplexed cucurbituril fall in the same range as for the adducts, but for the more rapidly tumbling unligated guests τ_c values are typically 4–10 ps. For the purpose of this article, the data are reduced to an *average correlation coefficient* for each complex, $\bar{\chi}$.[6,7] This is an average τ_c for the ^{13}C signals of the encapsulated guest divided by that of the host. The remarkable feature is the relative invariance of this parameter in comparison with the dissociation constants for the same complexes, which indicate a range of nearly 10000-fold in affinity between ligand and receptor (Table II, last column).

Table II. Rotational Correlation Times τ_c for Ligand–Receptor Complexes of **1**

ligand, τ_c (ps)[a]	$\bar{\chi}$[b]	10^6K_d, M
1. $CH_3(CH_2)_4NH_3^+$	0.14	42
receptor: 241, 246[c]		
ligand: $\beta\gamma$-CH_2 46, δ-CH_2 23[d]		
2. $H_3N^+(CH_2)_5NH_3^+$	0.16	0.41
receptor: 268, 274		
ligand: α-CH_2 34, β-CH_2 40, γ-CH_2 54		
3. c-$(CH_2)_3CHCH_2NH_3^+$[e]	0.14	2.7
receptor: 175, 161		
γ-CH_2 24		
4. c-[$(CH_2)_3OCH$]$CH_2NH_3^+$[f]	0.23	10
receptor: 316, 331		
ligand: α-CH_2 92, β-CH_2 61, $\gamma\delta$-CH_2 73, 92, ϵ-CH_2 67		
5. (2-C_4H_3S)$CH_2NH_3^+$[g]	0.26	4.3
receptor: 310, 283		
ligand: α-CH_2 72, $\gamma\delta$-CH 68, 91		
6. $C_6H_5CH_2NH_3^+$	0.11	3700
receptor: 323, 348		
ligand: α-CH_2 58, ArH 24, 33, 35		

[a] Values of τ_c for individual carbon atoms from T_1 measurements, estimated error ±20%. [b] Average correlation coefficient (ratio: av ligand τ_c/av receptor τ_c). [c] For CH and CH_2 carbons of **1**, respectively. [d] Positions α, β, γ, etc. in relation to ammonium substituent. [e] Cyclobutanemethylammonium ion. [f] 2-Tetrahydrofuranmethylammonium ion. [g] 2-Thiophenemethylammonium ion.

Discussion

Interpretation of the kinetic data in Table I is straightforward. A clear connection exists between the thickness of the ligand and its rate of complex formation with the receptor cucurbituril. The trend is succinctly illustrated by the cycloalkanemethylammonium ions, entries 5–7. The values of k_{in} are the more relevant numbers, since rates of dissociation (k_{out}) should reflect the different thermodynamic stabilities of the complexes, in addition to kinetic factors that we wish to evaluate. Cyclopentanemethylammonium ion, with a calculated maximum van der Waals diameter of 5.7 Å (i.e., across the 2,5-positions of the five-membered ring), forms its adduct most slowly. Cyclobutanemethylammonium ion, with a van der Waals diameter of 5.4 Å, binds more speedily by three orders of magnitude. Cyclopropanemethylammonium ion, diameter 4.6 Å, is even more rapid, as are the *n*-alkylammonium ions, diameter 4 Å.

This trend is rationalized by consideration of the interior dimensions of cucurbituril. As may be visualized from the cross-sectional representations (Figure 1), the center of the cavity of **1** has a van der Waals diameter sufficient to hold the larger substrates (a calculated interior dimension of 5.5 Å), but the portals represent a "bottleneck". The estimated internal van der Waals diameter of the occulus comprised of the six carbonyl oxygens surrounding each portal is only 4 Å (according to the crystallographic coordinates for **1**).[8] Clearly an *n*-alkyl hydro-

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(7) Kintzinger, J.-P.; Kotzyba-Hibert, F.; Lehn, J.-M.; Pagelot, A.; Saigo, K. *J. Chem. Soc., Chem. Commun.* **1981**, 833.

(8) Freeman, W. A. *Acta Crystallogr., Sect. B.* **1984**, *B40*, 382.

carbon chain may slip in without impediment, but the larger-dimensioned rings and isoalkyl hydrocarbon moieties (Table I, entries 1–5, all with diameters of ca. 5.7 Å and with similar values for k_{in}) are obliged to force their way into **1**, with probable distortion of the opening in the receptor.[9] We specifically note that relative rates of complex formation exhibit no correlation with stabilities of the adducts once formed (K_d). Explanation of variations within the latter has required a more sophisticated analysis.[4]

The limited number of cucurbituril complexes for which ^{13}C relaxation measurements are recorded (Table II) all exhibit similar low values for $\bar{\chi}$ (0.14–0.26). This indicates a lack of *dynamic cohesion* within the adducts; i.e., the substrate has considerable freedom for reorientation within the receptor. If the ligand were locked into the receptor, such that the molecular tumbling motions of both were synchronized, then a value for $\bar{\chi}$ near unity would be anticipated.[7] The apparent reason for an absence of mechanical coupling is the nearly cylindrical symmetry of cucurbituril, which allows the guest an axis of rotational freedom when ensconced within **1**. Hence, the bound substrates show only a moderate increase in τ_c relative to that exhibited in solution without **1**.

It is particularly noteworthy that no relationship exists between $\bar{\chi}$ and the thermodynamic stability of the complexes, as gauged by K_d. The first four entries in Table II represent C_5-alkylammonium ions. Relative to the *n*-pentyl case (1st entry), additional specificity features such as another $-NH_3^+$ group (2nd entry) or presence of a ring that is complementary to the interior of the receptor (3rd and 4th entries) provide no significant enhancement to $\bar{\chi}$. We have previously adduced evidence that a *benzene* ring is slightly oversized for the interior of cucurbituril.[3a] The benzylammonium ion adduct (6th entry) is about a thousand times less stable than the nearly optimally fitted thiophenemethylammonium ion complex (5th entry). A crystallographic study shows that incorporation of a benzene ring actually *distorts* the receptor into a ellipsoid shape.[8] It may be inferred that the high K_d value for benzylammonium ion reflects some *strain energy* (~4 kcal/mol) in the complex and that the ligand aryl ring is actually squeezed by the host.[3] Nevertheless, no enhancement of $\bar{\chi}$ results. It must be concluded that the interior of cucurbituril is notably "nonsticky". This reinforces our previous conclusions that the thermodynamic stability of the observed complexes of cucurbituril is chiefly governed by hydrophobic interactions of the solvated hydrocarbon components, plus electrostatic ion–dipole attractions between the carbonyls of the receptor and the ammonium cation of the ligands.

These results illustrate the advantages of working with a relatively inflexible, polycyclic receptor. A less rigid host, which can more easily accommodate itself to a ligand, might not be expected to exhibit such instructive dynamic behavior. Studies of cucurbituril may well demonstrate limiting-case properties for the types of specificity examined.

Experimental Section

Cucurbituril (**1**) was prepared by a modification of the procedure of Behrend.[10] The solvent for these investigations was 1:1 (v/v) HCO_2H–D_2O, in which **1** is quite soluble (10% solutions may be obtained). For the NMR (60 MHz) kinetic measurements, a displacing ligand (1,5-pentanediamine or 2-furanmethylamine, as required to minimize overlap of NMR signals) was added to a solution of one of the substrate complexes listed in Table I. Spectra were recorded at measured time intervals, and the appropriate integrals (vs internal reference *tert*-butyl alcohol) were fitted by nonlinear least-squares to an exponential decay function. Appropriate corrections were made in cases for which the back reaction could not be ignored with the displacing agents employed.[3a] As a demonstration of substitution mechanism, **1**·c-$(CH_2)_3CHCH_2NH_3$ exhibited only a 13(±19)% increase in exchange rate when the concentration of displacing agent (2-$C_4H_3OCH_2NH_3^+$) was increased 5-fold. For *n*-alkylammonium ions coalescense behavior was noted upon heating the NMR probe with 2:1 mixtures of substrate and **1**; the point at which separate signals merged was estimated visually. For the special case of cyclopropanemethylammonium ion, the analysis is as follows: The value of K_d ($=6.8 \times 10^{-5}$ M) ensures that there will be equal populations of bound and free guest when total RNH_3^+ substrate is taken in 100% excess. The difference in chemical shift for the two states amounts to $\Delta = 56$ Hz (from appropriate control measurements). Since the signals are coalesced at the temperatures of the other rate measurements (40 °C), it follows that $k_{out} > \Delta\pi/2 \simeq 124$ s^{-1} under the special circumstances described. Relaxation measurements (T_1) by ^{13}C FT–NMR spectroscopy (20 MHz) were secured conventionally.[6,7] Tolerances listed in this article are for the most part standard errors from least-squares analysis.

Acknowledgment. This work was supported by the Dow Chemical Co. Foundation, the University of Illinois Research Board, and in part by the Office of Naval Research.

Registry No. 1, 80262-44-8; $(CH_3)_2CH(CH_2)_2NH_3^+$, 62063-10-9; $(CH_3)_2CH(CH_2)_3NH_3^+$, 104464-49-5; 2-$C_4H_3SCH_2NH_3^+$, 119297-72-2; c-$(CH_2S)_2CHCH_2NH_3^+$, 119297-73-3; c-$(CH_2)_4CHCH_2NH_3^+$, 119297-74-4; c-$(CH_2)_3CHCH_2NH_3^+$, 119297-75-5; c-$(CH_2)_2CHCH_2NH_3^+$, 104464-67-7; $CH_3(CH_2)_3NH_3^+$, 16999-97-6; $CH_3(CH_2)_4NH_3^+$, 24551-46-0; $CH_3(CH_2)_5NH_3^+$, 21005-95-8; $CH_3(CH_2)_6NH_3^+$, 21005-96-9.

(9) Incorporation of a benzene ring is even slower: *p*-$CH_3C_6H_4CH_2NH_3^+$, $k_{in} = 2.75$ s^{-1} M^{-1} at 40 °C (extrapolated, ref 3).

(10) Behrend, R.; Meyer, E.; Rusche, F. *Justus Liebigs Ann. Chem.* **1905**, *339*, 1.