Molecular Spacewalk

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Dynamic Motion in Crown Ether Dendrimer Complexes: A "Spacewalk" on the Molecular Scale**

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Brownian motion, the rotation of molecules, and vibrations within molecules are typical forms of thermal motion. Fast chemical equilibria, such as the inversion at the ammonia nitrogen atom, the interconversion of conformers in alkanes, or highly dynamic association/dissociation processes in weakly bound noncovalent complexes are also thermally induced. In the context of noncovalent complexes, it is fascinating to examine whether an intracomplex migration of a guest molecule between different binding sites of a multitopic host is possible and how a motion like this could be monitored. Herein, the first five generations (**G1**–**G5**) of polyamino propylene amine (POPAM) dendrimers serve as prototypical multitopic hosts. We address the question, whether crown ethers can directly move from binding site to binding site on the dendrimers' periphery without intermediate dissociation/reassociation (Figure 1). Furthermore, if this molecular "spacewalk" is indeed possible, it raises the question as to by what mechanism it proceeds.

In solution, the detection of such an intracomplex binding-site hopping is challenging if not impossible, because it is always superimposed by dissociation/reassociation equilibria. Therefore, it is necessary to isolate the complexes from each other and from the corresponding free building blocks to suppress any intercomplex guest-exchange reactions. The high vacuum inside a mass spectrometer is ideally suited to achieve the isolation of the complexes as the complexes there are like-charged and thus efficiently separated from each other by charge repulsion. Also, reactions with neutral crown ether molecules can be excluded. Fragmentation of the crown ether/dendrimer complexes would be the only source for the appearance of neutral crown ethers in the gas phase. Therefore, their partial pressure is much too low to result in an efficient reattachment during the short time they spend inside the instrument before being pumped away. However, this approach comes with the difficulty that any intramolecular process does not change the complex ion's molecular mass and thus remains undetectable by a simple determination of the mass-to-charge ratio (m/z). Therefore, a gas-phase reaction is required that probes the guest's motion. Such a reaction must a) proceed energetically below the complex dissociation energy, b) cause a mass shift, and c) be directly linked to the guest movement.

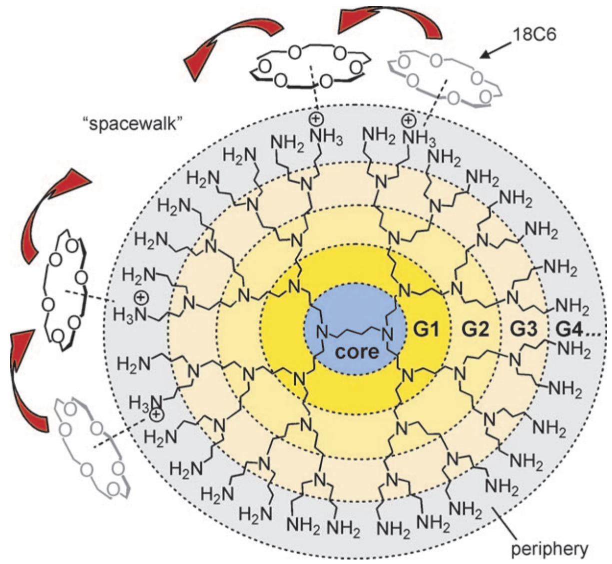

Figure 1. Chemical structure of [18]crown-6 and a fourth generation (**G4**) POPAM dendrimer. Starting with a 1,4-diaminobutane core, the *n*th shell of branches is divergently grown on the ($n-1$)th generation dendrimer by two Michael additions of acrylnitrile to each branch and subsequent hydrogenolytic reduction of the nitrile groups. The red arrows symbolize the main question of the present study: Can crown ethers move freely along the periphery of POPAM dendrimers without intermediate dissociation of the complex? As this process proceeds in the high vacuum inside a mass spectrometer, we refer to it as a molecular "spacewalk".

To realize this idea, we chose POPAM dendrimers as the multitopic scaffold. These dendrimers have highly branched onion-layer-type structures (Figure 1). From each generation (**G*n***) to the next, the number of peripheral amino groups doubles from four in the **G1** dendrimer to 64 in **G5**. Their gas-phase chemistry has been studied in detail.[1] In the absence of a solvating agent, protonation is likely to occur at interior tertiary amines rather than the peripheral primary NH_2 groups.[2] To examine the host–guest chemistry of dendritic molecules in the gas phase is generally a challenging and by-and-large unexplored field of research. Only a few examples exist to date.[3] In our study, [18]crown-6 serves as the guest, it binds to primary ammonium ions in solution,[4] and in the gas phase.[5] Dendritic crown ether/ammonium complexes are

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ideally suited for our purposes, because they are already charged and can easily be transferred into the gas phase as positively charged ions by electrospray ionization (ESI)[6] from slightly acidic solutions of the corresponding dendrimer and [18]crown-6 in methanol. A broad distribution of charge states (up to $z = +8$ for **G5**) with various stoichiometries (up to $n = 5$ [18]crown-6 molecules bound to **G5** is observed in the ESI mass spectra. Since crown ethers bind more strongly to primary (ca. 200 $kJ\,mol^{-1}$) than to tertiary ammonium ions (ca. 170 $kJ\,mol^{-1}$),[7] they provide solvation to the primary ammonium ions and thus contribute to shifting the charges to the peripheral amines.

Based on these considerations, we now can address the central question of this study: Can the crown ethers move from binding site to binding site at the periphery of a POPAM dendrimer? In its 1:1 complex with protonated ethylene diamine, [18]crown-6 (18C6) efficiently blocks the gas-phase exchange of the ammonium protons against deuterons [8] (Supporting Information), thus the H/D exchange reaction[9] is the reaction of choice to probe the crown ether's mobility on the dendrimer periphery: If it is unable to walk from binding site to binding site, the corresponding ammonium protons (i.e. three protons per crown ether) cannot be exchanged. In contrast, an exchange of all acidic protons on the dendrimer would be expected, when the crown ether moves.

For gas-phase H/D exchange experiments, the POPAM/crown ether complexes were generated in the ESI ion source and trapped in the collision hexapole of our Fourier-transform ion-cyclotron-resonance (FT-ICR) mass spectrometer.[10] Deuterated methanol (CH_3OD) was introduced into the hexapole through a time-controllable solenoid pulsed valve. The H/D exchange reaction is highly efficient[11] and all charge states and complex stoichiometries can be studied in the same experiment under the same conditions at the same time. After a well-defined reaction period, all product ions were transferred into the FT-ICR analyzer cell and detected with high resolution and mass accuracy (see Supporting Information).

As shown in Figure 2a, all nine N-centered protons of singly protonated **G1** can be exchanged quickly for deuterons. When a crown is bound to **G1**, three protons would be expected to be protected against the isotope exchange. In marked contrast to expectation, $[18C6@\mathbf{G1}+2H]^{2+}$ exchanges all its ten NH protons which is only possible if the crown ether has moved from one binding site to another during the exchange reaction. After 50 ms, the progress of the H/D exchange on the complex is more or less the same as that of free **G1**. Consequently, the crown ether movement proceeds with a rate more or less comparable to that of the isotope exchange. Remarkably, hardly any exchange is observed in the 2:1 complex $[(18C6)_2@\mathbf{G1}+2H]^{2+}$, which bears the same number of crown ether units and charges ($n = z$). This finding can be explained by a "relay" mechanism (Figure 2b) which has been postulated for crown ether complexes of ethylene diamine[8] and other small (bio)molecules.[12] As long as at least one ammonium group remains uncomplexed and thus freely accessible ($n < z$), the exchange reaction can operate efficiently according to the relay mechanism. When all charge sites are involved in crown ether binding instead ($n = z$), no ammonium group is available anymore to mediate the exchange. The exchange behavior observed for **G1** is confirmed in analogous experiments with higher generation dendrimers. For example, the isotope exchange on $[(18C6)@\mathbf{G2}+2H]^{2+}$ ($n < z$) is fast, while $[(18C6)_3@\mathbf{G2}+3H]^{3+}$ ($n = z$) proceeds at a much slower pace (Figure 2c).

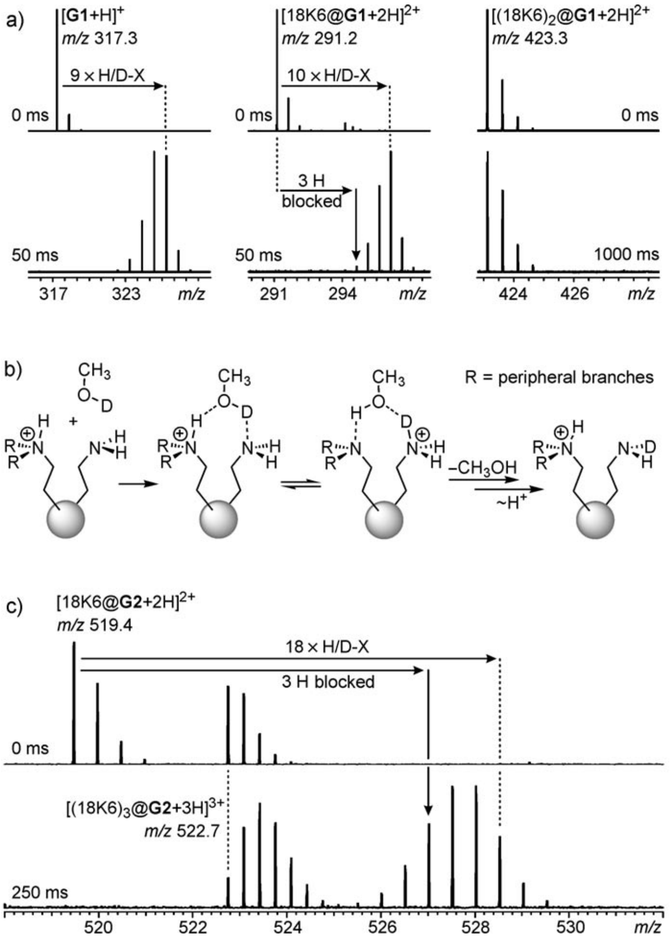

Figure 2. a) Left: H/D exchange (H/D-X) experiment with singly protonated **G1** (0 and 50 ms reaction time). Middle: H/D exchange of all ten NH protons of the doubly protonated 1:1 [18]crown-6/**G1** complex (0 and 50 ms). The vertical arrow marks the endpoint of the H/D exchange expected in case of a immobile crown ether unit blocking three protons against isotope exchange. Right: An extremely slow H/D exchange is observed for the doubly charged 2:1 [18]crown-6/**G1** complex (0 and 1000 ms). b) A "relay" mechanism[8,12] for the gas-phase H/D exchange at protonated POPAM dendrimers explains why the exchange is so slow in $[(18C6)_2@\mathbf{G1}+2H]^{2+}$. c) The same scenario is observed for higher generation dendrimers, see text for details.

For **G3**, **G4**, and **G5** dendrimer/crown ether complexes with their 16, 32, and 64 peripheral NH_2 groups, respectively, the number of signals in the mass spectra increases significantly. On the one hand, the number of different charge states and complex stoichiometries increases. On the other hand, defects in the dendrimer structure unavoidably accumulate owing to the divergent synthesis of POPAM dendrimers. Nevertheless, an unambiguous correlation of the signals after

H/D exchange to those prior to the experiment is easily possible by gradually increasing the reaction times. Each isotope pattern is then shifted step by step to higher m/z values so that the progress can be directly followed. Figure 3 depicts selected results for **G3** and **G4** with charge states up to $z=+7$ and $n=5$ crown ether units (see Supporting Information for data on **G5**): For all $(n<z)$ complexes, the H/D exchange clearly proceeds beyond the endpoint expected for complexes of immobile crown ether units (shown as solid vertical arrows). Even the complexes of **G4** and **G5**, which bear up to 71 and 136 exchangeable protons depending on their charge states, reach this threshold within less than 50 ms reaction time. Thus, we conclude the crown ether units to travel quickly on the dendrimer peripheries independent of the dendrimer size.

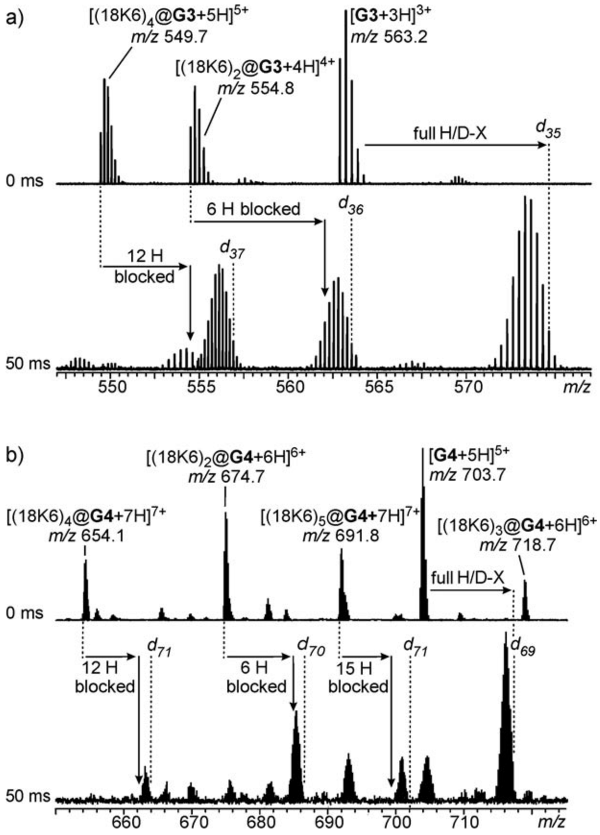

Figure 3. H/D exchange experiments conducted with [18]crown-6 complexes of a) **G3** and b) **G4**; d_y indicates the position at which full deuteration is obtained. Minor signals in the spectra are due to the typical defects in the dendrimer structure which unavoidably accumulate in the higher generations. See text for details.

The crown ether units' "spacewalk" may proceed according to two different mechanistic scenarios, though both proceed by the stepwise replacement one hydrogen bond after the other through a new one: either, the crown ether may be transferred as a neutral molecule from one ammonium group to another (Figure 4a); or, it could move together with a proton from its ammonium binding site to a neutral amine group (Figure 4b). With 1,12-diaminododecane (DAD; Figure 4c), a suitable and simplified model compound is available that allows to distinguish between both alternatives: a) A crown ether transfer within the doubly protonated 1:1 crown ether/DAD complex represents the ammonium-to-ammonium scenario in Figure 4a. When the crown ether transfer follows this mechanism, a complete exchange of all six acidic protons is to be expected, while the exclusive exchange of only three ammonium protons would provide evidence for a fixed crown ether and thus rule out the ammonium-to-ammonium scenario. b) The corresponding singly charged complex corresponds to the ammonium-to-amine alternative in Figure 4b. A full exchange again provides evidence for the transfer of a protonated crown ether from its ammonium site to the amine, whereas the exchange of merely two NH protons would rule out this scenario.

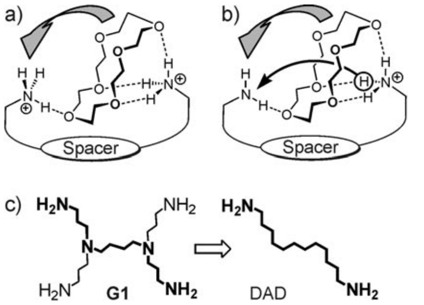

Figure 4. a, b) Schematic representation of both spacewalk mechanisms, see text for details. c) The model compound 1,12-diaminododecane (DAD).

The corresponding H/D exchange experiments (see Supporting Information) clearly rule out the ammonium-to-ammonium transfer and confirm the ammonium-to-amine scenario: In the doubly charged 1:1 complex, the three protons binding the crown ether remain unaffected by the exchange reaction. Consequently, no crown ether transfer occurs, probably because of the strong charge repulsion between the two ammonium ions. In contrast, all five protons can be exchanged in the singly protonated complex, however this reaction is comparably slow because of the absence of an additional free ammonium site that would facilitate the relay mechanism. This result clearly indicates a protonated crown ether moves from the ammonium to the amine terminus of the DAD chain. Hence, we conclude that the mechanistic scenario in Figure 4b prevails. For a full exchange of all the acidic protons, at least two crown ether transfer steps are therefore required: In the first step, the crown ether can move together with H^+ to an already deuterated ND_2 site resulting in a crown ether bound to an ND_2H^+ group. The isotope exchange of the last proton is only feasible, when the crown ether moves back—this time together with a deuteron.

Figure 5 depicts an alternative mechanistic scenario explaining the observed exchange of all labile hydrogen atoms without invoking crown ether transfers between binding sites. An adjacent terminal ammonium group or protonated tertiary amine in the dendrimer scaffold might form a proton bridge to one of the crown ether oxygen atoms. The binding energy gained helps to break an ammonium/crown ether proton bridge leading to a proton exchange. However,

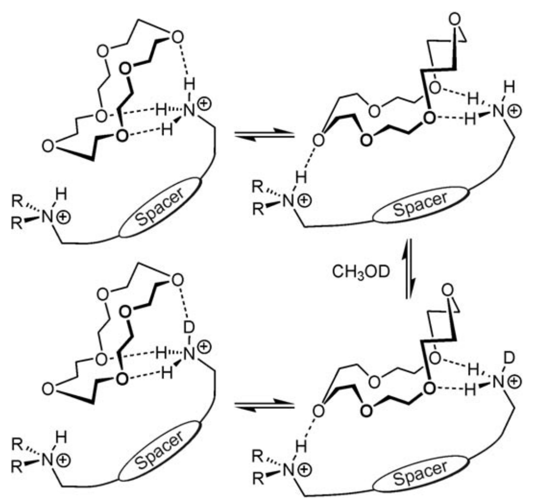

Figure 5. An alternative exchange mechanism, in which a nearby second charged site supports the release of an N–H⋯O hydrogen bond.

two reasons speak against such a scenario: 1) with longer spacers, such as the C_{12} chain in DAD, the generation of doubly charged complexes is possible —although quite some effort is required to optimize the ionization conditions. As the above results show, no complete exchange is observed in this complex ruling out the operation of the alternative mechanism, at least for longer spacers. 2) all attempts to generate doubly charged model complexes with shorter spacers, such as $[18C6@1,3\text{-diaminopropane}+2H]^{2+}$ failed. This result shows the significance of charge repulsion, which will not only affect ion generation, but also prevent the approach of the two like-charges in the gaseous complexes. For these reasons, we regard the crown ether transfer to be the more convincing mechanism.

In conclusion, an intriguingly simple experiment, that is, the H/D exchange conducted under the well-defined conditions in the high vacuum inside a mass spectrometer, is capable of monitoring the highly dynamic thermal movement of [18]crown-6 over the periphery of POPAM dendrimers irrespective of the dendrimer size (up to **G5**), charge state (up to +8), or the complex stoichiometry (up to five crown ether units). In the gas phase, the intracomplex binding-site hopping can be investigated without any interference from intercomplex transfers of crown ether units between dendrimer ions—thus making mass spectrometry the method of choice to investigate such a movement. In a more general sense, the crown ether spacewalk suggests that many non-covalent complexes—be they synthetic or biological supramolecules—may feature a significantly more pronounced dynamic behavior than frequently believed.

Experimental Section

All gas-phase experiments described herein were conducted with an Ionspec QFT-7 FT-ICR mass spectrometer (Varian, USA), equipped with a 7 T superconducting magnet and a Micromass Z-Spray electrospray ionization (ESI) source (Waters, France). Sample solutions (50 μM; in methanol, 1% formic acid) of [18]crown-6 and **G1**–**G5** POPAM dendrimer or 1,12-diaminododecane (DAD), respectively, were introduced into the ion source at flow rates of 2–4 μL min^{-1}. A constant spray and highest intensities were achieved with a capillary voltage of 3800 V and a source temperature of 40 °C. The parameters for sample cone and extractor cone voltages as well as the ion optics were optimized for maximum abundances of the desired complex ions. Multiple scans (up to 20) were averaged for each spectrum to improve the signal-to-noise ratio.

For the gas-phase H/D exchange experiments, we used the hexapole ion accumulation/collision cell of our instrument as an ion trap and reaction chamber for the isotope exchange reaction.[11] After ion accumulation in the hexapole, the entrance of new ions into the hexapole was blocked by switching off the radio frequency of the quadrupole in front of the hexapole (Supporting Information). To conduct the isotopic exchange, CH_3OD was then introduced into the hexapole. The reaction time was controlled with the help of a solenoid pulse valve which can be controlled with high temporal precision (steps of down to approximately 25 μs caused reproducible changes in the reaction progress). After the H/D exchange reaction, the ions were transferred into the instrument's FT-ICR analyzer cell and detected by a standard excitation and detection sequence, usually with baseline resolution for the isotope patterns of all charge states and high mass accuracy in the low ppm range.

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