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To cite this version:

HAL Id: hal-01259347
https://hal-amu.archives-ouvertes.fr/hal-01259347
Submitted on 20 Jan 2016

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High binding yet accelerated guest rotation within a cucurbit[7]uril complex. Toward paramagnetic gyroscopes and rolling nanomachines†

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The (15-oxo-3,7,11-triazadispiro[5.1.5.3]hexadec-7-yl)oxidanyl, a bis-spiropiperidinium nitroxide derived from TEMPO, can be included in cucurbit[7]uril to form a strong ($K_a \sim 2 \times 10^5$ M$^{-1}$) CB[7]@bPTO complex. EPR and MS spectra, DFT calculations, and unparalleled increased resistance (a factor of $\sim 10^3$) toward ascorbic acid reduction show evidence of deep inclusion of bPTO inside CB[7]. The unusual shape of the CB[7]@bPTO EPR spectrum can be explained by an anisotropic Brownian rotational diffusion, the global tumbling of the complex being slower than rotation of bPTO around its "long molecular axis" inside CB[7]. The CB[7] (stator) with the encapsulated bPTO (rotator) behaves as a supramolecular paramagnetic rotor with increased rotational speed of the rotator that has great potential for advanced nanoscale machines requiring wheels such as cucurbiturils with virtually no friction between the wheel and the axle for optimum wheel rotation (i.e. nanopulleys and nanocars).

Introduction

Molecular machines are increasingly being considered as promising architectures for advanced machineries proceeding at the nanoscale. Among them, nanocars are outstanding examples of such nanomachines where increased degrees of control are progressively gained over their design and their movements. However, all reports of such nanocars show the wheels covalently linked to the chassis implying friction problems upon movement. Among the macrocycles that could be used as wheels in a non-covalent strategy, cucurbiturils are rigid symmetrical round-shaped molecules with binding constants up to $7.2 \times 10^{17}$ M$^{-1}$. Here we show that suitably designed guests with high affinity can efficiently rotate in cucurbiturils with low friction. During the last two decades, the host–guest chemistry of CB[n] has been studied extensively using a combination of electronic absorption and NMR spectroscopy, mass spectrometry, and X-ray crystallography. In the past few years, EPR spectroscopy has also been used as an additional tool to explore the binding properties of CB[n] with paramagnetic molecules, containing one or several nitroxide moieties as probes. Lucarini first showed that TEMPO can be complexed by CB[7] ($K_a \sim 25 \pm 2 \times 10^3$ M$^{-1}$), the free and complexed radical exchanging slowly on the EPR time scale, and the latter showing smaller nitrogen hyperfine splitting and larger $g$ factor values ($\Delta\alpha_N = 0.11$ mT, $\Delta g = 0.0008$). Kafier et al. showed that the TEMPO moiety of 4-amido-2,2,6,6-tetramethylpiperidine-1-oxyl)cobaltocenium is engulfed in CB[8] to form a very stable inclusion compound ($K_a = 2.1 \pm 1 \times 10^8$ M$^{-1}$). The binding of one and two CB[8] macrocycles has been used to allosterically regulate the extent of spin exchange coupling in paramagnetic molecules bearing several nitroxide moieties. At concentrations above $10^{-3}$ M, an interesting selective aggregation of three supramolecules of nitroxide@CB[8] could be detected by EPR with various nitroxides. The three supramolecules are arranged in a triangular geometry that leads to spin exchange between the three radical centers. No such aggregation was evident in the case of CB[7] complexes. Nitroxide probes are widely used to investigate biological systems, however their use in vivo is often limited by their rapid reduction to EPR silent...
Various approaches have been developed to obtain nitroxide probes with increased resistance to bioreduction. One strategy to protect nitroxides from bioreductants is to include them into macrocycles such as cyclodextrins (CD). We and others have shown that the half-lives of various stable nitroxides or persistent nitroxide spin adducts can indeed be enhanced in the presence of CDs. However, because of the relatively weak binding constants of CD@nitroxide complexes, reductants, such as glutathione (GSH) or ascorbate, still remain active. Recently, we reported that CB[7] is a promising candidate in protecting the TEMPO nitroxide in the presence of an excess of ascorbate.

However, limitations still remain, due to the inherent dynamic inclusion complex equilibrium that leaves a fraction of the nitroxides exposed to the reductants. Recently different authors reported that a high degree of size and shape complementarity, and the presence on the guest of two positive charges, both positioned to interact with the CB[7]’s ring carbonyl oxygens through ion–dipole interactions, can lead to unprecedented CB[7]-guest affinity, with values (up to $K_a = 7.2 \times 10^{17} \text{M}^{-1}$) higher than that of the avidin–biotin pair.

Based on these results, we designed nitroxides (2,2-dimethyl-4-oxo-1,9-diazaspiro[5.5]undec-1-yl)oxidanyl PTO, and bPTO, having in water one or two protonated amine functions prone to position near the two carbonyl laced portals, and to force the N–O’ group to stand near the center of the CB[7] or CB[8] cavity (Scheme 1). Compared to TEMPONE we found that the binding affinities of bPTO for CB[7] and CB[8] are significantly increased, and once complexed bPTO becomes particularly resistant to reduction with ascorbate. Moreover, the EPR spectra of CB[7]@bPTO and CB[8]@bPTO complexes have a rather unusual shape. The high field line of the $^{14}\text{N}$ triplet is not broadened, as predicted due to the expected longer correlation time of the complexes compared to free bPTO. This behaviour can be explained by an anisotropic Brownian rotational diffusion, the global tumbling of the complexes being slower than the rotation of bPTO along its “long molecular axis” inside CB, the CB (stator) with the encapsulated bPTO (rotator) behaving as a supramolecular paramagnetic molecular rotor. Our results are presented and discussed hereafter.

**Results and discussion**

PTO and bPTO were prepared in a three-step sequence; experimental details for reaction procedures and characterization are given in the ESI. All the experiments were performed in water, and with a $pK_a$ of piperidine around 11.2, we will consider for the following discussion that PTO and bPTO are protonated at the amine sites.

**Mass spectrometry**

High resolution mass spectra of equimolar solutions of bPTO and CB[7] (1 mM) in water showed one peak at $m/z$ 708.2644 corresponding to a doubly charged cation of the formula $C_{55}H_{66}N_{31}O_{16}^{2+}$ which is in agreement with the composition $[(\text{CB7})\text{bPTO}]^{2+}$. Similarly with CB[8], the detection of a cation at $m/z$ 791.2893 corresponding to the formula $C_{61}H_{72}N_{35}O_{18}^{2+}$ is in agreement with a complex of the composition $[(\text{CB8})\text{bPTO}]^{2+}$.

**EPR characterization**

EPR spectra of PTO and bPTO show a typical three line pattern with a width at half height of 0.26 mT and 0.33 mT respectively and nitrogen coupling constants $\alpha_N$ of 1.57 and 1.53 mT respectively ($g_{\text{PTO}} = 2.0058$, $g_{\text{bPTO}} = 2.0061$, Fig. 1).

With regard to TEMPONE (0.08 mT), the larger linewidth observed for bPTO is mainly due to additional long range hyperfine couplings with $\gamma$ and $\delta$-hydrogens (see the ESI).

**Scheme 1** Structures of TEMPONE derivatives and of cucurbit[n]urils.
For bPTO, in the presence of CB[7], the nitrogen hyperfine coupling constant decreases significantly (ΔaN = 0.12 mT) and the g factor increases (Δg = 0.0006), in agreement with the formation of a CB[7]/bPTO inclusion complex, which is accompanied by the N–O’ group localization in the less polar surrounding of the CB[7] cavity. Usually, together with changes in aN and g values, the formation of a nitroxide inclusion complex is accompanied by a broadening of the EPR high field line (ΔI), resulting from an increase of the correlation time.\(^{15,16}\)

This broadening was not observed with CB[7]/bPTO (Fig. 1), and as discussed below this result can be accounted for by an anisotropic rotational diffusion tensor for the included bPTO.

EPR titration experiments were performed recording a series of EPR spectra obtained by gradually increasing the CB[7] or CB[8] concentrations. Using a 2D simulation program,\(^49\) binding constants \(K_a \sim 9 \times 10^7 \text{M}^{-1}\) and \(2.8 \times 10^5 \text{M}^{-1}\) were determined (Table 1) for the complexation of PTO with CB[7] and CB[8] respectively. The significantly smaller \(K_a\) value obtained for CB[7] is presumably due to steric hindrance at the PTO carbonyl-nitroxide region (O–O’ distance \(\approx 7.7 \text{ Å}\)) with van der Waals radii with respect to the cavity of CB[7] (entrance \(\approx 5.8 \text{ Å}, \text{ inner part } \approx 7.8 \text{ Å}\)). For bPTO, due to the presence of two piperidinium rings the affinity for CB[7] and CB[8] is expected to be higher. It reached \(1.8 \times 10^6 \text{M}^{-1}\) for CB[7] and was estimated (because we are close to the limit of reliable quantitative estimation of binding using EPR) to be above \(10^6 \text{M}^{-1}\) for CB[8]. The best fit between experimental and calculated EPR spectra was obtained assuming the formation of 1:1 complexes. Reduction experiments of the N–O’ group in the presence of ascorbic acid were performed (i) as an indication of the accessibility of the nitroxide function and (ii) as a way to determine the shielding effect, i.e. the efficacy of cucurbiturils to enhance the lifetime of nitroxides in biologically relevant media. Ascorbic acid was selected because it is known to be one of the most powerful reductants of nitroxides in biological fluids or cells, leading to very fast decay of their EPR signals in biological systems.\(^{26,50}\) We first monitored the EPR signals of the included bPTO (0.1 mM) in CB[7] and CB[8] (0.35 mM) after addition of ascorbic acid (2 mM). Over 90 minutes, the signal decay was very slow while at the same ascorbic acid concentration, the nitroxide alone is instantaneously reduced (Fig. 2).

### Table 1

<table>
<thead>
<tr>
<th>Nitroxide Complex</th>
<th>(a_N/\text{mT})</th>
<th>(K_a/\text{M}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPO</td>
<td>1.61</td>
<td>—</td>
</tr>
<tr>
<td>TEMPO/CB[7]</td>
<td>1.54</td>
<td>(\sim 10^3)</td>
</tr>
<tr>
<td>TEMPO/CB[8]</td>
<td>1.53</td>
<td>40</td>
</tr>
<tr>
<td>PTO</td>
<td>1.57</td>
<td>—</td>
</tr>
<tr>
<td>PTO/CB[7]</td>
<td>1.50</td>
<td>(9.0 \times 10^3)</td>
</tr>
<tr>
<td>PTO/CB[8]</td>
<td>1.45</td>
<td>(2.8 \times 10^5)</td>
</tr>
<tr>
<td>bPTO</td>
<td>1.33</td>
<td>—</td>
</tr>
<tr>
<td>bPTO/CB[7]</td>
<td>1.41</td>
<td>(1.8 \times 10^5)</td>
</tr>
<tr>
<td>bPTO/CB[8]</td>
<td>1.40</td>
<td>(&gt;10^6)</td>
</tr>
</tbody>
</table>

Interestingly, α-cyclodextrin (α-CD 50 mM), β-cyclodextrin (β-CD 10 mM), γ-cyclodextrin (γ-CD 100 mM) and 2,6-di-O-methyl-β-cyclodextrin (DM-β-CD 200 mM) that also show signs of inclusion of bPTO (Fig. 3a) afforded no protection, and no EPR signal could be detected 45 seconds after the addition of...
the reductant. These results show that CB[7] and CB[8] behave as effective shields around bPTO, and indicate that the N–O' group is deeply immersed in their cavity. We previously showed that CB[7] (12.75 mM, 100-fold excess) improved the protection of TEMPO (0.1 mM) regarding ascorbate reduction (2 mM), increasing its half-life to 254 minutes. The protection is much more efficient for bPTO (0.2 mM) with CB[7] (0.35 mM). The intensity of the CB[7]@bPTO EPR lines is reduced by only ~23% after 16 hours which corresponds to an approximate t1/2 of ~17 h. Because under the same experimental conditions, the half-life time of bPTO alone is <1 min, complexation with CB[7] affords a ca. 105-fold enhancement in the protection of the N–O' group. The results obtained using CB[8] (t1/2 ~ 21 h, ESI†) are very similar to those found with CB[7].

Rotational dynamics

EPR studies. Inclusion of bPTO inside CB macrocycles is not accompanied by the usual broadening of the EPR high field line (I/Io = 0.897 and 0.964 for bPTO and CB[7]@bPTO respectively; I, Io, and I are the peak-to-peak amplitudes of the low-field, central and high-field line respectively, Fig. 1). To the best of our knowledge, it is the first time a slight increase of I/Io is observed after the formation of a CB@nitroxide inclusion complex. Different studies have shown that for a nitroxide the relative peak-to-peak amplitudes depend strongly on the rotational dynamics. In order to get more details on this process, EPR spectra were fitted with the EasySpin routine chilii and home-written Matlab scripts. The results for CB[7]@bPTO are discussed hereafter, and those concerning CB[8]@bPTO are given in the ESI.† Two types of fits were performed. For the first type, the whole spectral line shape was fitted with fixed 14N hyperfine and fits were performed. For the first type, the whole spectral line field line (not accompanied by the usual broadening of the EPR high field line that is larger than can be achieved with this motional model or any model that we tested. The best approach to this high relative intensity is obtained if motion about the x and z axes is much slower (740 ps) than that about the y axis.

Our results show that for CB[7]@bPTO the host and the guest have no dynamic cohesion. As previously mentioned by Mock, the reason for the absence of mechanical coupling is the nearly cylindrical symmetry of cucurbiturils which allows guests to keep an axis of rotational freedom. However, usually the rotational motion of the complexed guest becomes restricted due to steric constraints. For bPTO, slowdown of rotational diffusion about the x and z axes on inclusion into CB is expected because of the larger effective radius of the particle undergoing rotational diffusion. The fact that rotational diffusion about the y axis remains fast and actually appears to speed up is unexpected. The data strongly suggest that bPTO rotates about its long axis after inclusion into CB with less friction than in pure water. This increase of rotational motion around the y axis could be reasonably accounted for by the successive formation of the same set of hydrogen bonds, between ammonium hydrogen atoms and the CB portal carbonyl groups, requiring a small energy barrier as in molecular ball bearings. In order to get more evidence on the rotational dynamics, we also examined the changes (Fig. 3) in the EPR spectra of bPTO and bPyTO, in the presence of a large excess of 2,6-di-O-methyl-β-cyclodextrin (DM-β-CD) and CB[7] respectively.

In both cases, the observed decrease of &al and the broadening of the high field line are in agreement with the formation of an inclusion complex (ΔΔal = 0.07 mT for DM-β-CD@bPTO, and ΔΔal = 0.1 mT for CB[7]@bPyTO). Spectral calculations using the first type of fit indicated again the absence of dynamic cohesion. Interestingly, in the absence of either the CB[7] carbonyl groups for DM-β-CD@bPTO or the ammonium ions for CB[7]@bPyTO (Fig. 3b), calculations predicted a slowing down (Table S1 and Fig. S23†) of the rotational dynamics after complexation.

We want to point out that the actual CB[7]@bPTO motion may be more complex, and the relative line intensities in the fast motion regime may not provide enough information for fully characterizing it. However, both types of EPR fits indicate a speed-up of rotational motion around the y axis, and we believe that the values from the first type of fit are more realistic than the extreme speed-up found with the second type of fit.

DFT calculations. Although crystal structures of cucurbituril inclusion complexes are generally possible to obtain, we were unsuccessful in getting crystals of CB[7]@bPTO and CB[8]@bPTO. DFT calculations were performed assuming that in these complexes, bPTO could adopt three main conformations (trans-trans, trans-cis and cis-cis) that differ in the geometry of the spiro junctions in regard to the N–O’ bond (Scheme 2). For all the calculated conformers of the complexes, the two ammonium groups interact with the CB’s ring

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Nanoscale

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12146 | Nanoscale, 2015, 7, 12143–12150

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Molecular dynamics calculations. Molecular dynamics (MD) simulations in water, over 100 ns period, were performed for CB[7]@[bPTO] using Gromacs 5.0.4 package (see details in the ESI†). The results indicate that during the trajectory, the nitroxide guest stays deeply included in the cavity of CB[7] in agreement with EPR and DFT results. The distance between the CB[7] geometric center and the nitrogen atom carrying H20 (one ammonium hydrogen atom, Fig. 5a) is nearly constant (4.4 ± 0.4 Å, Fig. 5c), and the distance between H20 and the O1 oxygen atom of CB[7] oscillates between 2 and 7 Å (Fig. 5a). These results show that during a trajectory (i) the position of bPTO does not change significantly along the C7 axis of the macrocycle (ii) bPTO rotates around the y-axis (Scheme 1) with the N–O′ group remaining almost located in the plane passing through the CB[7] equatorial hydrogens. In agreement with this rotation, the angle θ between vectors V1 and V2 (respectively defined by the N–O and C–H bonds in Fig. 5b) takes all the values between 0° and 180°. Fig. 5e shows the distribution of θ values over two 100 ns trajectories, starting from θ = 0° and θ = 180° respectively. Interestingly, the value in between maxima is about 50°, an angle which corresponds to jumps of the ammonium hydrogen atoms from one carbonyl oxygen to another by steps ~2π/7 (Fig. 5e).

There are few studies reporting guest rotational dynamics in molecular containers. Because guests were reported to have slower dynamics when included in cucurbiturils, the present acceleration of guest rotation upon binding was unexpected and represents an alternative solution to the oligo-ketone guest proposed by Keinan for a “lowered-friction” molecular rotary motor. We think that the present jumping model, where the hydrogen bonding ammonium function moves almost freely by increments of nearly 50°, is due to preorganization of the CB[7] carbonyl crown where the ketone oxygen atoms are ready to hydrogen bond (on both sides of the CB) thus lowering the barrier to jump from one ketone to the next. In this view, the multiply hydrogen bonded network of bulk water (solvent shell) certainly plays a role because the two ammoniums of bPTO are less solvated when included in CB[7]. Such a solvent vs. preorganized macrocycle effect has already been reported for related systems such as in lubricated molecular shuttles, ring rotations within catenanes, or in simple N-aryl imide molecules. Still the present results will prove to be useful in the design of advanced CB[n] based molecular machines like supramolecular gyroscopes and molecular ball-bearings. More generally, the present guest design offers new perspectives for any application requiring fast-spinning wheels where cucurbiturils can be used, and also critical spinning information can be obtained from the free radical labelled guest and EPR spectroscopy.

Conclusion

Reduction of nitroxides is recognized to be one of the main limitations for their use in biology. We have shown that sequestration with high affinity in CB[7] or CB[8] of suitably-
designed nitroxides can dramatically improve their resistance to reduction (lifetime of several hours with minor decay in the presence of 20 fold excess of ascorbate). Our results could open new perspectives to the use of nitroxides in biological milieu. Additionally, our results highlight the advantages of cucurbiturils as stators offering restricted friction for optimized rotational motion in tailored molecular rotors. Such non-covalent molecular rotors can open new avenues toward nanoscale molecular machines on which one could exert control over the rotator for fast spinning movements such as nanopulleys and all nanoscale machines where pulleys are involved or for the construction of small motor vehicle chassis such as nanomotorcycles or nanocars. In particular, the present study highlights the great potential of cucurbiturils for the construction of CB-wheeled nanocars. Work along this line is in progress and will be reported in due course.

Acknowledgements

The CNRS, Aix-Marseille Université, Renard RPE TGE and Région PACA (project “Masked Spins”) are acknowledged for financial and technical support. We also thank Laszlo Jicsinszky (Cyclolab), Sébastien Combes and the CRCMM, ‘Centre Régional de Compétences en Modélisation Moléculaire de Marseille’ for computing facilities.

Notes and references
