Supramolecular Chemistry

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Shaping Micelles: The Interplay Between Hydrogen Bonds and Dispersive Interactions**

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Micelles are globular aggregates, usually formed by amphiphilic molecules in water;[1–3] they have often been used to stabilize membrane proteins for further studies,[4] or in applications in drug delivery,[5,6] industrial processes,[7] and catalysis.[8] In this last application, reverse micelles are more commonly employed. In a reverse micelle, the polar groups are pointing inwards, and the nonpolar groups are in contact with the solvent.[9,10]

As micelles are formed by a limited number of molecules, their behavior is midway between the bulk phase and the atomic world.[11,12] These molecular aggregates are thus useful probes of the intermolecular forces controlling aggregation and pose multiple fundamental questions connecting structure and function. Most notably: Are micelles self-sustained substrates, or do they require a solvent? What is the minimum size of a molecular cluster displaying micellar characteristics? And finally, what is the balance of non-covalent forces controlling aggregation? To shed light on these issues, we have applied a particularly uncommon reductionist approach for the construction of a micelle: we grew isolated intermolecular clusters of controlled size, sequentially increasing complexity in the cold and collision-free environment of a supersonic expansion.

This isolated-molecule description prevents any solvent effect, thus exactly revealing the intramolecular factors producing self-association. Micelles are formed by weak intermolecular forces, so they are usually dynamic objects that might adopt different configurations. Many studies have been published about the forces that drive the formation of these molecular aggregates, but the issue remains an unsolved problem.[13–15] The absence of solvent in our study is vital to discern the contribution of such weak interactions to the global stability of the aggregates.

We use supersonic expansions of a mixture of a nucleation molecule and a noble gas to create the molecular aggregates inside the vacuum chamber of a linear time-of-flight mass spectrometer.[16] In this work, micelles were formed from propofol, probably the most widely used general anesthetic, both because of our previous knowledge of its spectroscopy, and because it combines moderately polar and aliphatic groups. The cold environment of the expansion preserves molecular clusters on a microsecond timescale, which allows us to carry out a laser spectroscopy study,[17] while the stoichiometry of the aggregates is partially controlled. Accurate structural data can be obtained by electronic, double- or triple-resonance laser spectroscopy techniques,[18] which are directly comparable with the predictions of quantum chemical calculations.[19] Such a combination has been previously applied to small intermolecular clusters, but has seldom been used for molecular aggregates of more than three or four molecules.[17]

Figure 1a shows the mass spectrum of an expansion of propofol/He (UV laser off resonance, so as to not modify the original abundance of the species). Clustering more than three monomers is experimentally challenging for molecules of this size, and only a few references can be found describing the spectroscopy of trimers or larger systems.[20] By integrating the signal in each mass channel while the UV laser is scanned, it is possible to record the resonance-enhanced multi-photon ionization (REMPI) spectrum of the $S_1 \leftarrow S_0$ electronic transition of each species with vibrational resolution (Figure 1b). The REMPI spectrum of propofol contains contributions from four different conformational isomers, as previously demonstrated,[21,22] which differ in the relative orientation of the isopropyl groups. Likewise, two conformational isomers were found for the propofol dimer; both of them with C–H···π interactions as the main cohesive forces.[16]

The REMPI spectrum of the propofol trimer is quite clean, and presents a very high s/n ratio, whereas the UV/UV double-resonance experiment demonstrates that the spectrum is due to a single isomer. Adding more propofol molecules results in the appearance of a broad absorption, the importance of which increases until it dominates the spectrum of the propofol hexamer.

Using the IR/UV double resonance technique, which allows the mass-resolved IR spectrum of the aggregates to be obtained selectively,[23] important structural information can be obtained from each cluster. Figure 2 shows the IR/UV

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Supporting information for this article, including experimental set up and calculation procedures, is available on the WWW under http://dx.doi.org/10.1002/anie.201303245.

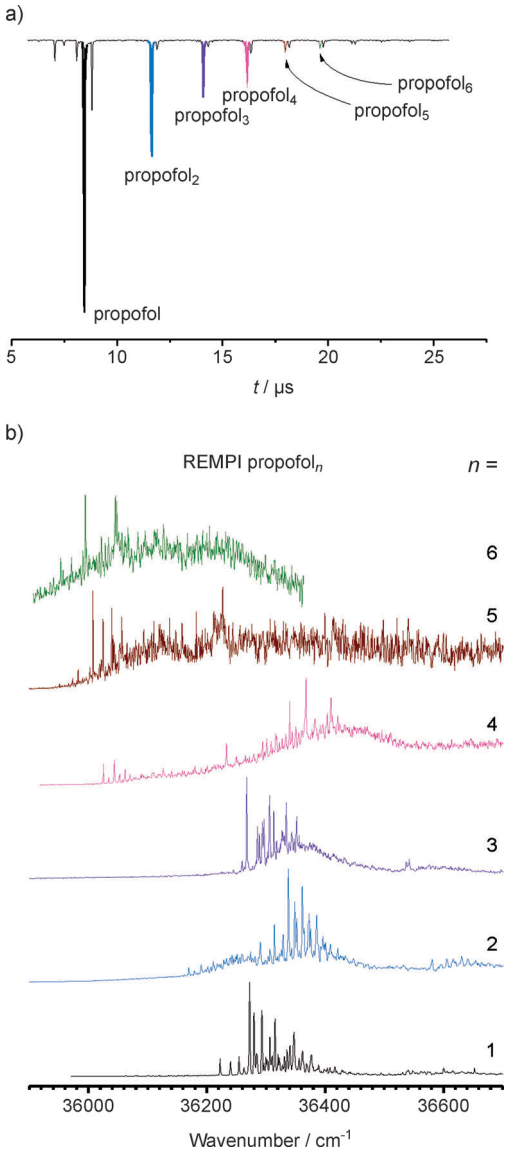

Figure 1. a) Mass spectrum of the supersonic expansion of a mixture of propofol in He. b) REMPI spectrum of each species in the expansion, obtained by integrating each mass channel while the probe laser is scanned.

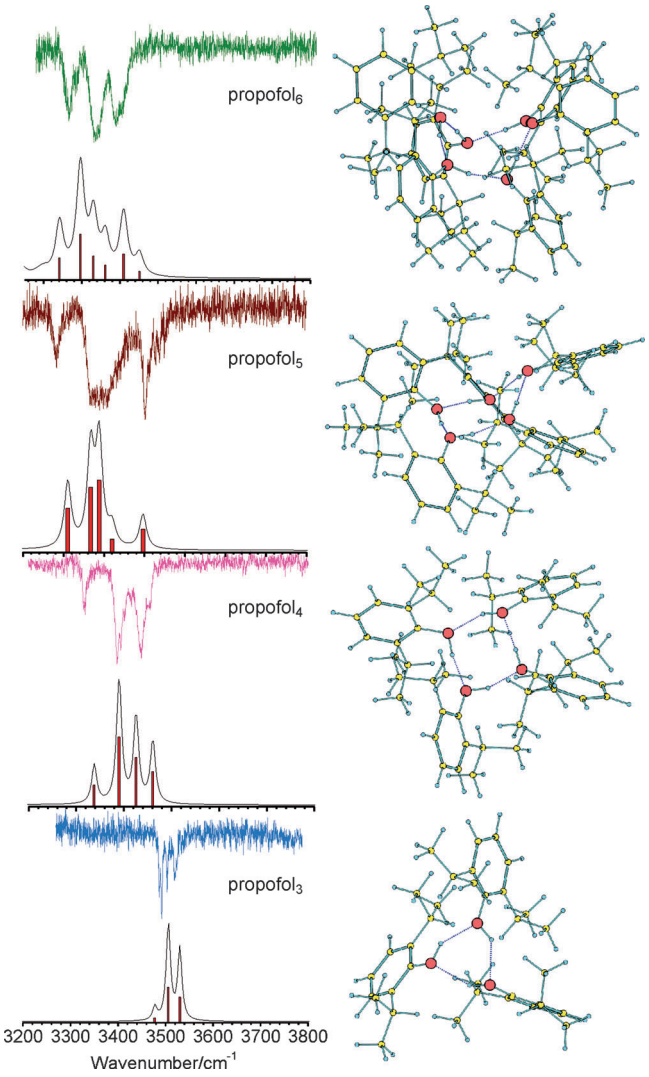

Figure 2. IR/UV double resonance spectra of propofol homoclusters containing 3–6 propofol molecules, together with the predicted spectra for the structures to which the cluster is assigned. The final structure assigned to each complex is presented to the right of the IR/UV spectrum. H blue, C yellow, O red.

spectra of the main conformers of the aggregates of propofol containing 3–6 molecules. When probing different transitions of the UV spectrum, more than one isomer was found for the tetramer and pentamer, but they are omitted here for the sake of brevity, as their assignment leads to the same conclusions as the assignment of the strongest conformers.

Propofol has a single OH bond, and therefore a single band is expected for each propofol molecule in the aggregate in the 3100–3800 cm^{-1} region. Accordingly, three bands are found in the IR/UV spectrum of the trimer, whereas three broader bands and a shoulder are present in the spectrum of the tetramer. The increase in the width of the bands may indicate a reinforcement of the hydrogen bonds with the inclusion of an additional propofol molecule. Such behavior is a well-known indicator of the formation of cooperative hydrogen-bond networks. Furthermore, all of the transitions are shifted to the red from the single band of the propofol monomer, once more indicating the existence of a hydrogen bond network. As can be seen, all of the bands appear in a very narrow interval of wavelengths, which points to the formation of hydrogen bonds of similar strength. There is a general shift to the red in the bands as the size of the cluster increases.

Although five transitions are expected in the spectrum of the propofol pentamer, only three bands were resolved, but the broad central band may hide contributions from several vibrational transitions. Finally, only three bands (grouped around 3200 cm^{-1}) were resolved in the spectrum of the propofol hexamer, thus indicating that there are three pairs of equally strong hydrogen bonds, which points to the existence of some symmetry in the molecule.

To aid in the interpretation of the IR/UV spectra, the conformational landscape of the clusters was explored

through a combination of molecular mechanics and quantum mechanical methods. A priori, a system of this size may adopt hundreds (or even thousands) of structures of very similar stability. However, the sharp, well defined lines in the spectra indicate that the aggregates adopt discrete conformations. The systems presented in this work are among the largest ever studied using this combined experimental and theoretical approach, and are of considerable size from the calculation point of view, taking into account that quantitative predictions are required to assign the experimental spectra. All of the calculated structures are collected in Figures S1, S3, S5, and S7 in the Supporting Information, whereas those whose predicted spectra better reproduce the experimental results are collected in Figure 2. A remarkably good agreement between calculated and experimental spectra is found, as is demonstrated by the comparison in Figure 2. As can be seen, all of the structures present cyclic hydrogen-bond networks formed by the interaction between the OH groups. The high directionality of the hydrogen bonds imposes a frame on the aggregates, over which the rest of the interactions, mainly C–H···π, are built, thus giving the cluster its final dynamic shape. In this way, the trimer presents a six-membered hydrogen bond network, with all of the aromatic rings interacting on one side of the aggregate, whereas the OH moieties are exposed on the other side. The simulated spectrum faithfully reproduces the experimental spectrum, confirming the assignment.

Regarding the propofol tetramer, it is hard to a priori imagine a structure that is in agreement with the experimental observations: as previously stated, the IR spectrum clearly shows a reinforcement of all the hydrogen bonds formed by the OH groups, which is indicative that a cyclic structure is adopted by all of the OH groups, as happens in pure water clusters. However, the two bulky isopropyl groups at both sides of the OH moiety hamper formation of hydrogen bonds between two propofol molecules. Such substituents may be envisioned as two big hydrophobic arms protecting the OH group. However, the propofol tetramer shows a surprisingly simple and elegant structure: the two OH moieties form an eight-membered cyclic hydrogen-bond network, whereas the aromatic rings are positioned in such a way that the isopropyl group of one molecule interacts with the aromatic rings of the neighboring molecules. The result is a kind of molecular fan (see animations in the Supporting Information). Such a structure allows the molecules to maximize both hydrogen bonds and dispersive forces.

Calculations on the propofol pentamer show that the structure whose predicted spectrum best reproduces the experimental spectrum is globular (Figure 2; see the Supporting Information for all predicted spectra). This globular structure has a diameter of 1.4 nm and a hydrophilic heart composed of a cyclic hydrogen bond network surrounded by a hydrophobic shell. As can be seen, all of the OH groups take part in the formation of a pentagonal hydrogen-bond network. This reinforces the hydrogen-bond network, both because of the increase in the number of participants on the one hand and the aperture of the OH···O angles, which are now close to linearity (Figure S9), on the other. The shape of the hydrogen bond network closely resembles that of the envelope conformation of cyclopentane (Figure 3), because the oxygen atoms are sp^3 hybridized, as are the carbon atoms of cyclopentane. For the same reason, the hydrogen bond network is not planar. In cyclopentane, this configuration

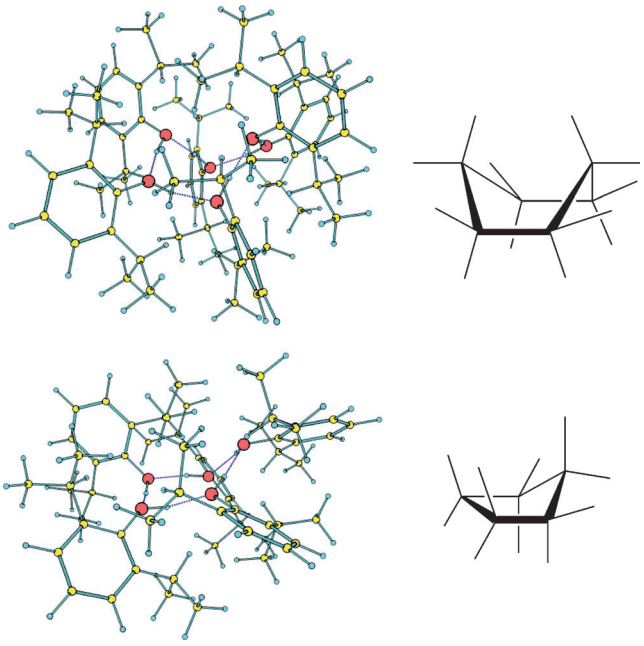

Figure 3. Comparison of propofol pentamer and hexamer structures (left), and the envelope and boat conformations of cyclopentane and cyclohexane (right). H blue, C yellow, O red.

avoids eclipsing of the hydrogen atoms, which would otherwise decrease the stability by 41.8 kJ mol^{-1}.[24] However, in the propofol pentamer, the cluster gains additional stabilization from the dispersive interactions between the isopropyl groups and the aromatic rings of the neighboring molecules, which slightly distorts the envelope shape.

Following the trend observed for the tetramer and pentamer, the calculated propofol hexamer which better reproduces the experimental spectrum presents a hexagonal hydrogen bond network (Figure 2) that resembles the boat conformation of cyclohexane (Figure 3). Although the chair cyclohexanol is more stable than the boat conformation, in the case of propofol hexamer the latter allows the cluster to maximize C–H···π interactions. These are among the weakest interactions,[25] but their presence in large number in the hexamer gives the aggregate extra stabilization. The hydrogen bonds appear in pairs of similar bonding distance and angle (Figures 4 and S9). Nevertheless, the structure of the experimental cluster must be more symmetrical, as indicated by the overlapping of some of the vibrations in the IR spectrum.

Figure 4 shows the distances and angles adopted by the OH moieties in the propofol pentamer and hexamer. As can be seen, the latter reverse micelle has a radius of 1.58 nm, which makes it the smallest micelle observed and fully characterized to date. In an excellent paper on the cetyltrimethyl–ammonium bromide system using electrospray ionization and mass spectrometry, Sharon et al. have already found evidence of the survival of reverse micelles in the gas

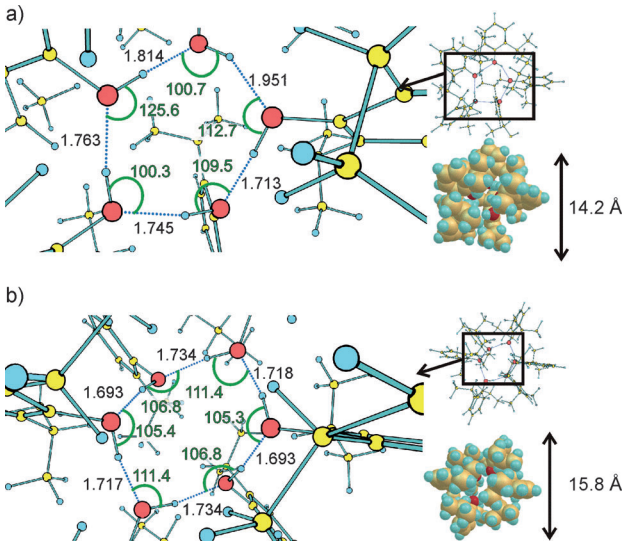

Figure 4. Angles and distances of the hydrogen-bond network in propofol pentamer (a) and hexamer (b), together with the diameter of the cluster. H blue, C yellow, O red.

phase.[26] We complement such results here with the fully resolved structure of a reverse micelle in gas phase.

In summary, this work clearly highlights the importance of the contribution of several weak non-covalent interactions, such as hydrogen bonding and dispersive interactions, which are well-known to play a fundamental role in the final shape of biological supramolecular structures. Certainly, a micelle may be envisioned to be one of the most primitive forms of self-organization and may therefore be related to the appearance of life on earth.[27] For the aggregates analyzed in this work, the highly directional hydrogen bonds build a frame on top of which the dispersive forces give the aggregate its final shape. Such an observation may be also extrapolated to proteins and other superstructures. Thus, the N–H···O=C interactions between the peptidic bonds give rise to the well-known α-helix and β/γ-turns commonly observed in proteins, whereas the rest of the non-directional interactions add to give them their final shape.

Regarding the questions at the beginning of this manuscript, this work demonstrates that solvent is not needed to create a micelle, at least under expansion conditions. Furthermore, the micellar nucleus, composed of the hydrogen-bond core, is already present in the propofol trimer. The rest of the monomers simply pile up, adding hydrogen bonds to the network and forming the hydrophobic shell.

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