Medium Effects in Action, Visualized by the Crystal Structures of Open and Closed Forms of a Molecular Receptor

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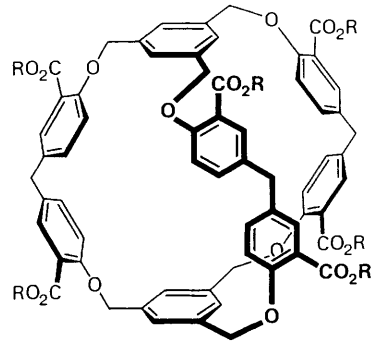
The water-soluble macrobicyclic cyclophane molecule 1^{6-} has been crystallized in two very different shapes that display the operation of medium, especially hydrophobic effects: an inflated cage structure building up cylinders disposed in an hexagonal array; a flattened structure stacked in molecular layers separated by aqueous layers in a lamellar arrangement.

Medium factors are thought to have a marked influence on the structural features of solutes. In particular, hydrophobic effects[1] are expected to affect strongly the behaviour of organic molecules in aqueous solution. Thus, amphiphilic substances generate a range of supramolecular arrangements in aqueous solution in order to segregate hydrophilic from hydrophobic structural subunits.[2–4] On the other hand hydrophobic effects also play a very important role in determining the tertiary and quaternary structures of biomolecules.[5]

Macropolycyclic molecules of a cyclophane type[6,7] can be made water soluble by the introduction of polar groups. Such amphiphilic cyclophanes may function as molecular receptors for various substrates in aqueous solution. Modification of their shape by hydrophobic–hydrophilic effects could markedly affect their substrate binding properties. It is thus of great interest to gain information about medium effects on molecular shape.

We have recently described[8] the synthesis and the substrate-binding properties in aqueous solution of the macrobicyclic molecule 1^{6-}. Receptor 1^{6-} contains a hydrophobic core fitted



1 R = Na
1^{6-} R = –

† UPR 285 of the CNRS.

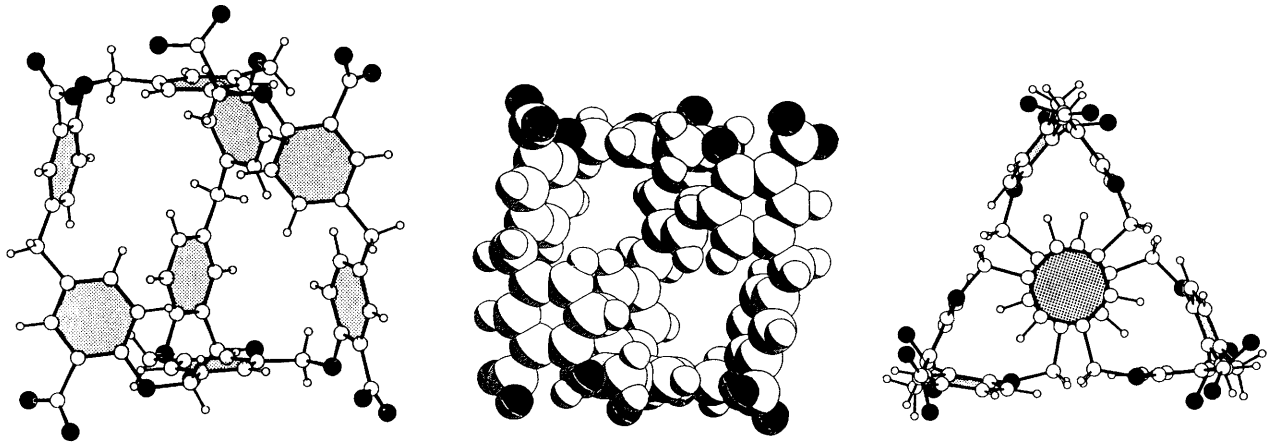


Fig. 1 Inflated shape of molecule 1^{6-} in crystal structure (*I*). (Left): ORTEP representation with the long axis of the molecule vertical; (centre): space-filling model with the same orientation; (right): view along the ternary axis.

with six hydrophilic carboxylate groups that make it water soluble; it binds strongly a variety of cationic organic substrates in aqueous solution. We now report that the sodium salt **1** has been crystallized in two different forms that provide direct insight into an illustration of the operation of hydrophobic effects on molecular shape. The results also give a close-up observation in the 'frozen' crystalline state of solute–solvent ordering processes in fluid phase.

Two different types of crystals (*I*) and (*II*) were obtained by crystallization of the sodium salt (1^{6-}, $6Na^+$) under different conditions.‡ They display two strikingly different shapes and solvation states for the macrobicyclic cage 1^{6-}.

Crystals (I). The unit cell contains 2 receptors and 16 localized solvent molecules, characterized as 8 methanol and 8 water molecules. One methanol molecule is identified in disorder inside the cage. The macrobicycle, located on the crystallographic 6_3 axis, has a ternary symmetry. The cations are distributed between general and special positions with partial occupancy.

The molecule 1^{6-} is represented in Fig. 1. It has an open, barrel-type shape, with distances of 9.2 Å between the top and bottom terminal phenyl rings and of 10.6 Å between the methylene bridge carbons, thus forming a large cavity of 150 to 200 $Å^3$, which can contain up to 10 guest species (water or methanol). These molecules (except one methanol molecule) were not localized and must be moving freely inside the barrel.

The cage 1^{6-} is chiral, in the shape of a triple helix, with a 90° twist angle. All the carboxylate groups, bearing the negative charges, are turned outwards. The molecules are piled along the 6_3 screw axis forming infinite cylinders, with an intermolecular separation of 3.4 Å between stacked terminal phenyl rings. The carboxylate groups are arranged in negatively charged doughnuts, regularly spaced along the molecular helix (every 12.7 Å), each molecule being rotated by 180° with respect to the preceding one (Fig. 2, left). The carboxylate groups point outwards, below and above the terminal phenyl rings, in a staggered position with respect to those of the consecutive molecule and are linked together through sodium ions.

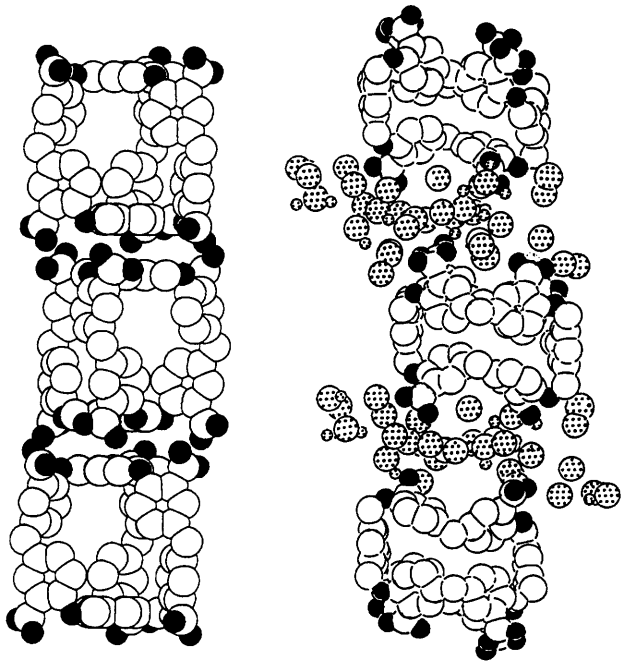


Fig. 2 Representation of: (left) the stacking of the inflated form of 1^{6-} into cylinders (surrounded by solvent molecules and Na^+ ions) as found in structure (*I*); (right) the stacking of the flattened form of 1^{6-} into molecular layers separated by layers of solvent molecules and Na^+ ions (large and small dotted circles) as found in structure (*II*); note the position of the terminal phenyl rings compared to the figure on the left.

The infinite parallel molecular helices form a hexagonal array of columns immersed in a medium of sodium ions and water molecules (Fig. 3, left). The carboxylate groups make strong external bonds to the cations and water molecules located in the channels. Other sodium cations, with their polyhedron of coordinated oxygens, are situated in the channels formed by these parallel columns.

Crystals (II). There are 2 identical macrobicycles, 28 water and 2 methanol molecules per asymmetric unit. The shape of the two ligands and the solvent environment are completely different from those observed in crystal (*I*).

‡ *Crystal data* for (*I*), hexagonal; $P6_3$; a = 13.604(6), c = 25.36(1) Å; 39 atoms refined; R = 7.5%, were obtained from a solution of the hexasodium salt 1^{6-}, $6Na^+$ in a mixture of water, methanol and THF. *Crystals* (*II*), monoclinic; *Cc*; a = 37.803(15), b = 15.525(6), c = 30.36(1) Å, β = 121.39(2)°, 206 atoms refined; R = 8.3%; in the presence of 2 equivalents of adamantane amine hydroiodide, very small crystals were obtained which were checked by NMR to contain the ligand and the substrate; recrystallization gave suitable crystals (*II*) for X-ray analysis; however, these crystals (*II*), although different from crystals (*I*), contained again only the uncomplexed hexasodium salt 1^{6-}, $6Na^+$ together with methanol and water molecules. Structures were solved by direct methods.[11] Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

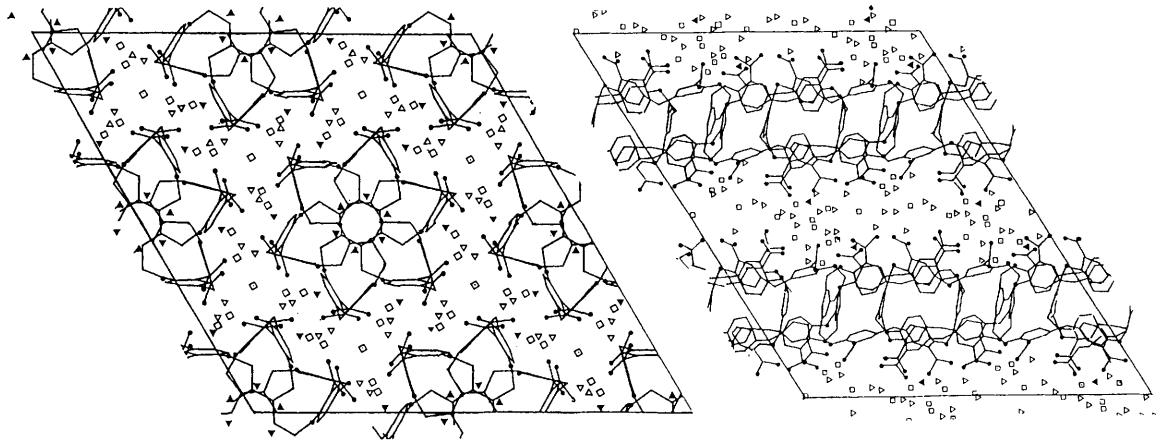


Fig. 3 Crystal packings. (Left) Hexagonal packing of the molecular cylinders formed by stacks of the inflated form of 1^{6-} immersed in (solvent + Na^+) as found in structure (*I*); projection along the *c* axis of the content of 4 unit cells. (Right): Lamellar packing of monomolecular layers of the flattened form of 1^{6-} separated by thick layers of water molecules and sodium ions as found in structure (*II*).

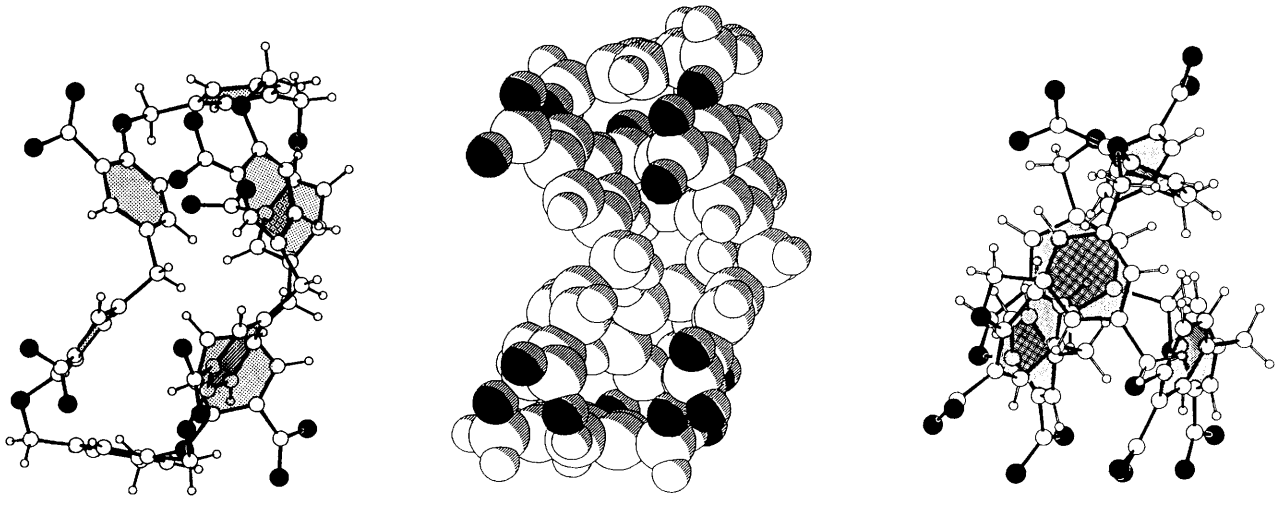


Fig. 4 Flattened shape of molecule 1^{6-} in crystal structure (*II*). (Left): ORTEP representation with the molecular axis vertical; (centre): space-filling model with the same orientation; (right): view perpendicular to the first two.

The cage 1^{6-} is flattened, elongated, with distances of 11.3 Å between the terminal phenyl rings, and of only 4.5 Å between the methylene bridge carbon atoms (Fig. 4). The three bridges are in van der Waals contact, two of them having their phenyl rings nearly parallel (dihedral angle between 10 and 19°). This face-to-face stacking narrows one 'window' of the macrobicyclic structure down to 4.0 Å (mean phenyl separation) (Fig. 4, right). The two other 'windows' are wide open, giving a flattened shape to the molecule. No internal void is observed.

The different 1^{6-} units are in contact only through their hydrophobic core. The crystals are formed by two-dimensional arrays of such stacked molecules, the organic layers being separated by thick aqueous layers (Fig. 2, right; 3, right). Thus, the ligands form infinite monomolecular layers, with all the negative charges borne by the carboxylate groups spread on both surfaces of the layer. These molecular sheets are separated by an organized net of solvent molecules (MeOH and H_2O) wrapping the sodium ions, the methanol molecules being, as in structure (*I*), rather situated on the envelope defined by the carboxylates. The carboxylate groups, located on the two opposite sides of the organic layers, stick into the aqueous layers formed by 30 solvent molecules and 12 sodium ions per unit cell. Thus, these layers represent a concentrated aqueous solution of sodium ions containing for each sodium ion two carboxylate oxygens and about 2.5 water molecules, which is below the usual solvation/hydration number of these ions.

The packing features observed in the present crystal structures bear relation to those of species formed by amphiphilic molecules: a hexagonal structure in which the molecules are stacked in cylinders arranged in hexagonal packing and separated by the aqueous medium forming a honeycomb; a lamellar structure consisting of an alternate stacking of organic layers, containing the hydrophobic core of the molecules, and aqueous layers. In both cases (*I*) and (*II*), the polar head groups are the hydrophilic carboxylate functions, sticking out, at the interface of the two media, of isolated barrel-shaped molecules in (*I*) and of the other surfaces of the molecular layers in (*II*). These layers also represent a model picture of the membranes formed by bolaamphiphiles.

Structure (*I*) bears some resemblance to the structure of a γ-cyclodextrin–propan-1-ol inclusion complex containing 17 water molecules.[9] On the other hand, structure (*II*) might be compared with that of calix[4]arene sulfonate which in the

solid state combines with sodium ions and water molecules to produce an ordered double layer arrangement.[10]

The inflated (*I*) and flattened (*II*) structures of molecule $\mathbf{1}^{6-}$ clearly illustrate how medium effects affect molecular shape and how this shape is related to the supramolecular organization of the medium. As noted above, the occurrence of two highly different structures for the same molecule $\mathbf{1}^{6-}$ may be compared with the behaviour of amphiphilic molecules which can organize into different periodic structures depending on the conditions. In the specific case of $\mathbf{1}^{6-}$, the ability to function as a receptor molecule for various substrates is expected to be influenced by the operation of medium factors such as hydrophobic effects that will deform the shape of the receptor. These factors come into play when functional molecules are incorporated into membrane phases as well as in the determination of the structure and function of biomolecules.

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References

1 C. Tanford, *The Hydrophobic Effect*, Wiley, New York, 1973; *Science*, 1978, **200**, 1012.

2 V. Luzzati, in *Biological Membranes*, ed. D. Chapman, Academic Press, London, 1968.

3 A. Skoulios, *Ann. Phys.*, 1978, **3**, 421; J. Charvolin and J.-F. Sadoc, *La Recherche*, 1992, **23**, 307.

4 J. H. Fendler, *Membrane Mimetic Chemistry*, Wiley, New York, 1982.

5 H. Meirovitch, S. Rackovsky and H. A. Scheraga, *Macromolecules*, 1980, **13**, 1398; H. Meirovitch and H. A. Scheraga, *Macromolecules*, 1980, **13**, 1406; R. S. Spolar, J.-H. Ha and M. T. Record, Jr., *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 8382.

6 J. Frank and F. Vögtle, *Top. Curr. Chem.*, 1986, **132**, 135; C. Seel and F. Vögtle, *Angew. Chem.*, 1992, **104**, 542, *Angew Chem., Int. Ed. Engl.*, 1992, **31**, 538; Y. Murakami, J. Kikuchi and T. Ohno, *Adv. Supramol. Chem.*, 1990, **1**, 109.

7 F. Diederich, *Angew. Chem.*, 1988, **100**, 372; *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 362; *Cyclophanes*, Royal Society of Chemistry, Cambridge, 1991.

8 R. Méric, J.-P. Vigneron and J.-M. Lehn, *J. Chem. Soc., Chem. Commun.*, 1993, 129.

9 J. Ding, T. Steiner and W. Saenger, *Acta Crystallogr., Sect. B*, 1992, **47**, 731.

10 A. W. Coleman, S. G. Bott, S. D. Morley, C. M. Means, K. D. Robinson, H. Zhang and J. L. Atwood, *Angew. Chem.*, 1988, **100**, 1412; *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1361.

11 G. Sheldrick, SHELX76, A program for Crystal Structure Determination, University of Cambridge, 1976.