JOURNAL OF PHYSICAL ORGANIC CHEMISTRY, VOL. 11, 693–696 (1998)

Complexation of basic amino acids by water-soluble calixarene sulphonates as a study of the possible mechanisms of recognition of calixarene sulphonates by proteins

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Received 14 May 1997; revised 14 November 1997; accepted 17 November 1997

ABSTRACT: The interactions of calixarene sulphonates with the basic amino acids arginine and lysine were studied by ^{1}H NMR spectroscopy. Strong electrostatic binding occurs for calix[4]arene sulphonate with both lysine and arginine at pH 1 and 5. For the higher calixarenes, only weak interactions at the faces of the flattened macrocycles occur. This binding is in contrast to the inhibition of protein–protein interactions by the calixarenes where the calix[6]arene and calix[8]arene sulphonates show much stronger effects. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: calixarene sulphonates; amino acids; proteins

The calix-arenes[1] are the third major class of supramolecular host systems along with the crown ethers[2] and the cyclodextrins.[3] Their ease of production and relative simplicity of chemical modification have produced increased interest in their chemistry over the last few years. A resorcinol-derived calix-arene has been shown by Schneider *et al.*[4] to form complexes with organic ammonium ions. In addition, the electrostatic binding of quaternary amines to water-soluble calixarene sulphonate derivatives has been investigated by Morozumi and Shinkai;[5] in particular, the inclusion complex formed from *p*-sulphonated calix[4]arene and trimethylanilinium was studied by ^{1}H and ^{13}C NMR spectroscopy.[6] However, in spite of extensive early work of the biological properties of the *p*-sulphonated calixarenes,[7] it is only very recently that interest in their biomedical potential has come to the fore again.[8] The charge density and size of the *p*-sulphonated calixarenes make them excellent candidates as heparin mimics, particularly in view of the difference in the synthetic pathways to the sulphonated derivatives (three steps)[9] as compared with the heparin pentasaccharide (over 50 steps).[10] We have noted this heparin mimicry with respect to peptide folding and even protein–protein interactions.[11] In this work, we investigated the fundamental bases of the interactions between *p*-sulphonated calix[4]arene (**1**), calix[6]arene (**2**) and calix[8]arene (**3**) with the basic amino acid residues arginine (**4**) and lysine (**5**), known for their electrostatic anchoring of the heparin fragment[12] (Fig. 1).

The study used ^{1}H NMR titrations (Varian 500 MHz instrument) in 95% H_2O–5% D_2O under non-buffered conditions and at pH 1 (adjusted with hydrochloric acid because sulphuric acid is competitive with the calixarene sulphonate groups), 5 and 13 (adjusted with sodium hydroxide). Buffering cannot be used here as **1, 2** and **3** are all capable of interacting strongly with many common buffers.

Compounds **1, 2** and **3** were synthesized using the method described by Arena *et al.*[13]

The results are given in Fig. 2(a)–(d) for the chemical shifts of the amino acid protons versus the relative concentration of the amino acids. For clarity only the spectral changes for **1** and lysine and arginine at pH 1 and

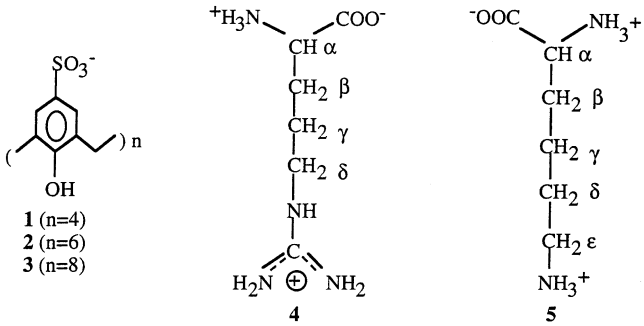

Figure 1. Structure of p-sulphonated-calix[4]arene (**1**), calix[6]arene (**2**), calix[8]arene (**3**), arginine (**4**) and lysine (**5**)

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CCC 0894–3230/98/100693–04 $17.50

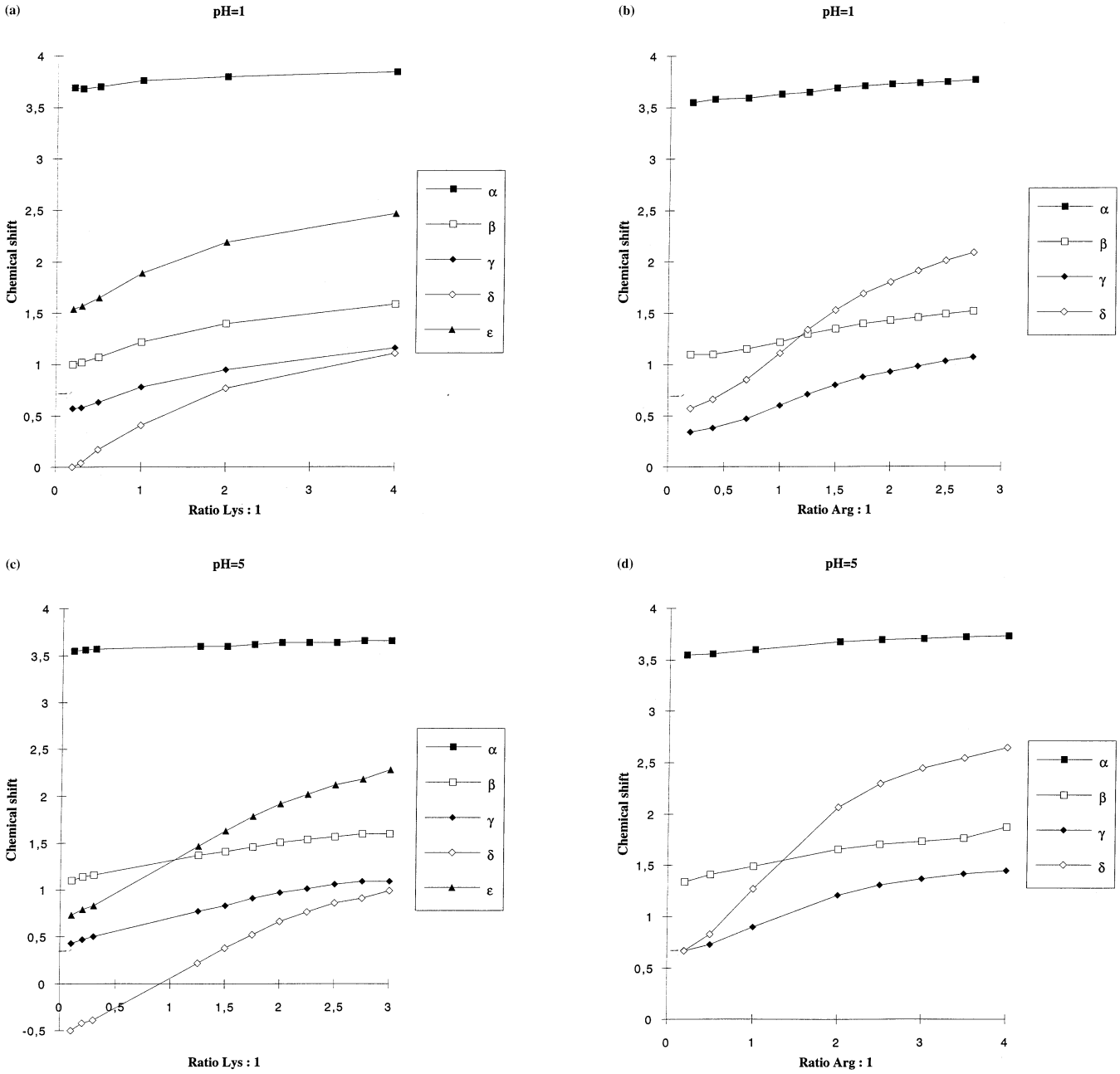

Figure 2. Chemical shift values for (a) lysine protons as a function of the lysine: **1** ratio at pH 1, (b) arginine protons as a function of the arginine: **1** ratio at pH 1, (c) lysine protons as a function of the lysine: **1** ratio at pH 5 and (d) arginine protons as a function of the arginine: **1** ratio at pH 5.

5 are given. As would be expected from purely electrostatic considerations, no interactions are observed at pH 13, as here the guest does not carry positive charges to bind to the negative charges on the host.

In the case of **2** and **3,** small variations (0–0.5 ppm between the complexed form of the amino acid and the free form) for lysine and arginine are observed at pH 1 and particularly pH 5. These can be ascribed to weak electrostatic interactions between the amino acids and the host systems. As shown by Gutsche and Bauer,[14] **2** and **3** adopt an increasingly planar conformation, and their very flexible geometries do not present a predefined host cavity as found for **1**.[15] Hence only simple binding to the negatively charged faces will occur.

It was found previously that in aqueous solution **1** adopts a cone conformation,[16] probably, fixed as a result of very strong intramolecular hydrogen bonding involving the O^- and OH groups of the phenolic units.[17] At pH 1 and 5, the binding of **1** to lysine and arginine causes large changes in the chemical shifts of the protons of the amino acid lateral chains. All peaks shift to higher magnetic field with increasing calixarene concentration.

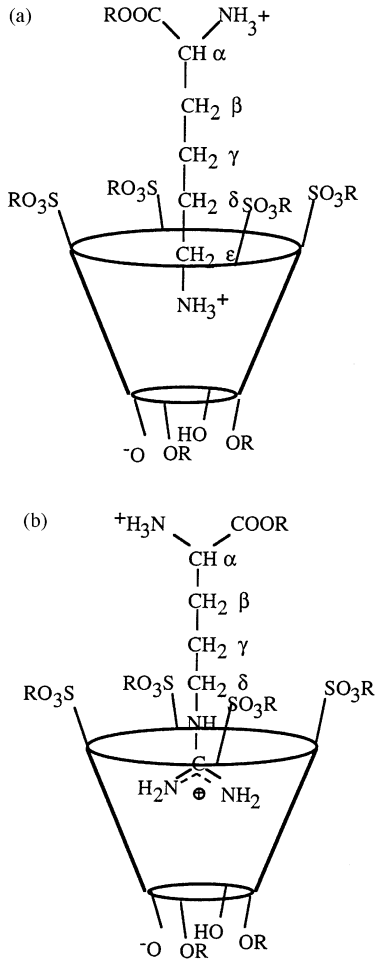

Figure 3. Schematic diagrams of the complexes between **1** and (a) lysine and (b) arginine (pH = 1, R = H; pH = 5, R = –).

The shifts increase along the chain, indicating that the amino acid is included in the cavity of **1** and affected by the ring current of the aromatic components [Fig. 3(a) and (b)].

The use of molecular modelling (ALCHEMY) showed that the cavity of **1** can contain the terminal group of arginine and the lateral chains of the two amino acids.

The stoichiometry is 1:1 for all complexes, as confirmed by Job's plots. The derived binding constants were evaluated for the arginine δ protons and lysine ε protons: **1**–Lys (pH 1) = 100, **1**–Arg (pH 1) = 200, **1**–Lys (pH 5) = 600 and **1**–Arg (pH 5) = 1700. The binding constants for arginine are considerably higher than for those lysine, arising from π–π interactions between the guanidinium functions and the aromatic groups of **1.**

In the region between pH 1 and 4, the shift changes decrease slightly owing to increasing ionization of the carboxylate function of the amino acids; however, in the region of pH 4, the phenolic OH functions of **1** are ionized and there is a sharp increase in the chemical shift changes.

The complexation is essentially driven by electrostatic effects. Titrations carried out in the presence of metal salts (K^+, Na^+, Mg^{2+} and Ca^{2+}) at high metal concentrations (>100 mM) essentially show no chemical shifts changes. This is typical for non-specific electrostatic interactions between ligands and peptide receptors. There is therefore pH-selective recognition of **1** for the amino acids with much stronger binding under neutral than acidic conditions. This has important biological implications; low pH values are found in the stomach and vesicles whilst pH 6–7 is the physiological pH. There can be expected to be switching between recognition sites on protein surfaces by **1** which will now be dependent on the biological localization.

Hence two distinct mechanisms of binding of positive amino acid residues to the *p*-sulphonated calix arenes exist, tight inclusion in the case of **1** and weaker face-to-face residue binding for **2** and **3.** Such different binding mechanisms will translate into different biological effects on the binding to heparin receptor peptides. These are long sequences, either α-helical[18] or β-sheet,[19] with separations of up to 20 Å between blocks of positive charges,[20] in addition, the binding mechanisms often involve cross-linking of proteins or peptide sequences[21] which are spatially separated and act in an allosteric fashion in the heparin recognition process. Hence, even though the binding of **1** may be stronger, **2** and **3** may be expected to behave as better mimics for heparin. In fact, preliminary results concerning the inhibition of collagen XIV binding to the dermatan sulphate side-chain of decorin, a heparin-inhibited process,[22] show effects $\mathbf{1} < \mathbf{2} \approx \mathbf{3}$.[23]

Acknowledgement

N. Douteau-Guével gratefully thanks V. Kalchenko, A. Lesage and R. Montserret for their help.

REFERENCES

1. V. Böhmer. *Angew. Chem., Int. Ed. Engl.* **34,** 713–745 (1995).
2. G. W. Gokel. *Monograph in Supramolecular Chemistry: Crown Ethers and Cryptands*. Royal Society of Chemistry, Cambridge (1991).
3. J. Szejtli. *Cyclodextrin Technology*. Kluwer, Dordrecht (1988).
4. H. J. Schneider, D. Güttes and U. Scheider. *Angew. Chem., Int. Ed. Engl.*, **5,** (1986);*J. Am. Chem. Soc.* **110,** 6449–6454 (1988).
5. T. Morozumi and S. Shinkai. *J. Chem. Soc., Chem. Commun.* 1219 (1994).
6. S. Shinkai, K. Araki, T. Matsuda, N. Nishiyama, H. Ikeda, I. Takasu and H. Iwamoto. *J. Am. Chem. Soc.* **112,** 9053–9058 (1990).
7. J. W. Conforth, E. D. Morgan, K. T. Potts and R. J. W. Rees. *Tetrahedron* **29,** 1659 (1973).
8. M. A. S. Pinhal, C. P. Dietrich, H. B. Nader, W. Jeske, J. M. Walenga and D. Hoppenstead. *Thromb. Haemostasis* **69,** 1247a (1993).
9. S. Shinkai, K. Araki, T. Tsubaki, T. Arimura and O. Manabe. *J. Chem. Soc., Perkin Trans I* 2297–2299 (1987); G. Arena, R. Cali, G. G. Lombardo, A. Casnati, E. Rizzarelli, D. Sciotto and R. Ungaro. *Supramol. Chem.* **1,** 19–24 (1992).

10. A. A. Van Boeckel and M. Petitou. *Angew. Chem., Int. Ed. Engl.* **32,** 1671–1690 (1993).
11. A. W. Coleman. paper presented at the 6th International Symposium on Inclusion Compounds, Istanbul (1995).
12. J. R. Fromm, R. E. Hileman, E. E. O. Caldwell, J. M. Weiler and R. J. Linhardt. *Arch. Biochem. Biophys.* **323,** 279–287 (1995).
13. G. Arena, A. Contino, G. G. Lombardo and D. Sciotto. *Thermochimi. Acta*, **264,** 1–11 (1995).
14. C. D. Gutsche and L. J. Bauer. *J. Am. Chem. Soc.* **107,** 6052–6059 (1985).
15. A. W. Coleman, S. G. Bott, S. D. Morley, C. M. Means, K. D. Robinson, H. Zhang and J. L. Atwood. *Angew. Chem., Int. Ed. Engl.* **27,** 1361 (1988).
16. S. Shinkai, K. Araki, T. Matsuda and O. Manabe. *Bull. Chem. Soc. Jpn.* **62,** 3856–3862 (1989).
17. S. Shinkai, K. Araki, H. Koreishi, T. Tsubaki and O. Manabe. *Chem. Lett.* 1351–1354 (1986).
18. R. St Charles, D. A. Walz and B. F. P. Edwards. *J. Biol. Chem.* **264,** 2092–2099 (1989).
19. R. Tyler-Cross, M. Sobel, D. Marques and R. B. Harris. *Protein Sci.* **3,** 620–627 (1994).
20. G. Venkataraman, V. Sasisekharam, A. B. Herr, D. M. Ornitz, G. Waksman, C. L. Cooney, R. Langer and R. Sasisekharan. *Proc. Natl. Acad. Sci. USA* **93,** 845–850 (1996).
21. S. Faham, R. E. Hileman, J. R. Fromm, R. J. Linhardt and D. C. Rees. *Science* **271,** 1116–1120 (1996).
22. B. Font, E. Aubert-Foucher, D. Goldschmidt, D. Eichenberger and M. Van der Rest. *J. Biol. Chem.* **268,** 25015–25018 (1993).
23. E. Aubert-Foucher. unpublished results.

