A γ-cyclodextrin duplex connected with two disulfide bonds: synthesis, structure and inclusion complexes†

Sergey Volkov,a Lukáš Kumprecht,a Miloš Buděšínský,a Martin Lepšíka,Michal Dušekb and Tomáš Kraus*a

Per(2,3,6-tri-O-benzyl)-γ-cyclodextrin was debenzylated by DIBAL-H to produce a mixture of C6I,C6IV and C6II,C6III isomeric diols, which were separated and isolated. The C2-symmetrical C6I,C6IV diol was transformed into thiold and dimerized to produce a γ-cyclodextrin duplex structure. A crystal structure revealed tubular cavity whose peripheries are slightly elliptically distorted. The solvent accessible volume of the cavity of the γ-CD duplex is about 740 Å3. Due to this large inner space the duplex forms very stable inclusion complexes with steroids; bile acids examined in this study show binding affinities to the γ-cyclodextrin duplex in the range of 5.3 × 107 M−1–1.9 × 108 M−1.

Introduction

Complexation of molecules and ions, both organic and inorganic, by receptors with high affinity and selectivity in an aqueous environment plays a key role in various biological processes. Chemists discovered a broad range of synthetic analogues that were designed to mimic these processes. Within macrocyclic receptors operating in aqueous media, cyclodextrins1 (CDs) play important roles due to their abilities to form host-guest complexes with a wide range of organic molecules. The binding of guest molecules inside CDs is assumed to be driven mainly by the hydrophobic effect (favorable solvation entropy due to the release of host-bound water molecules upon complexation) and van der Waals interactions between the guest and non-polar parts of the cavity.2 It was suggested that the free energy of binding in such host-guest systems is proportional to the surface area of the guest buried upon binding.3 Since cavities of CDs are relatively shallow (∼6–7 Å) they do not allow complete inclusion of molecules longer than one benzene ring preventing thus a strong binding unless additional attractive interactions, such as ionic4 or coordination5 bonding with suitably modified CD hosts, are involved. Consequently, binding affinities of native CDs mostly fall in the 102–103 M−1 interval1,4 limiting the scope of their applications in fields such as drug delivery systems or in supramolecular analytical chemistry,7 namely in fluorescent indicator displacement assays,8 operating at low concentrations in a complex biological environment.9,10

In order to improve binding affinities of CDs, dimeric constructs were prepared with the enlarged inner cavity by means of a connection of two CD macrocycles together via single or multiple linkers. Singly-bridged dimers11 revealed binding affinities significantly higher as compared to native CDs in some cases, yet the cavities acted independently12,13 in other cases due to the large flexibility of the single connection, yielding 2:1 complexes with usual stabilities known for native CDs. To prevent flexibility, rotational freedom of the two CDs was restricted by the introduction of additional linking groups. These molecules, termed duplex CDs14 (or CD duplexes15), were composed of α- or β-cyclodextrin units connected with two bridges of variable length and with varying mutual orientations of the bridged macrocycles.14–25 In addition, α-cyclodextrin duplexes connected with three26 and six27 linkers have been reported. Nevertheless, only few examples of significantly increased binding capabilities are known,25,26,28 supposedly due to non-optimal spacing of the two macrocyclic cavities or low solubilities of the duplexes.

We have recently described the syntheses and properties of new host systems composed of two α-CD or β-CD macrocycles linked with disulfide bonds.25,26,29–31 These tube-like molecules showed unusually high binding affinities (Kd up to 1010 M−1) to various organic compounds from hydrophobic α,ω-alkanediols25,26 to fairly hydrophilic medium-sized molecules such as imatinib.31 Now, we have turned our attention to a larger γ-CD homologue. In this work, we report the synthesis,
crystal structure and binding properties of a novel tubular receptor consisting of two $\gamma$-CDs linked with two disulfide bonds on their primary rims in a head-to-head manner designed for the complexation of larger organic molecules, such as steroids, in aqueous media.

Results and discussion

Synthesis

In our previous studies$^{25,31}$ on dimerization of $\alpha$-CD or $\beta$-CD macrocycles, the C6$^{I},$C6$^{IV}$-disulfanyl $\alpha$-CD or $\beta$-CD precursors were prepared by a sequence of synthetic transformations starting from perbenzylated $\alpha$-CD or $\beta$-CD that were selectively debenzylated at C6$^{I}$ and C6$^{IV}$ positions by a DIBAL-H promoted procedure.$^{22,33}$ This method allowed cleavage of benzylic groups in positions C6$^{I},$C6$^{IV}$ of perbenzylated $\alpha$-CD or $\beta$-CD macrocycles with high selectivities and yields. However, preparation of pure selectively bis(de-O-benzylated) $\gamma$-CD homologue(s) has not been reported yet. Thus, in order to prepare the required $C_2$-symmetrical C6$^{I},$C6$^{V}$-disulfanyl $\gamma$-CD we needed to develop a procedure for the preparation of C6$^{I},$C6$^{V}$-debenzylated $\gamma$-CD.

Starting perbenzyl-$\gamma$-CD 1 (Scheme 1) was prepared by exhaustive benzylation of anhydrous $\gamma$-CD with benzyl chloride in DMSO using sodium hydride as a base. Next, we investi-
gated the course of the debenzylation reaction at various concentrations of DIBAL-H as we had observed earlier \(^{29,31}\) with smaller \(\alpha\)-CD or \(\beta\)-CD homologues that more concentrated solutions allow smoother cleavage at or below room temperature. We found that 3 M solution of DIBAL-H in toluene allowed partial control over the extent of debenzylation allowing the isolation of a mixture of products containing perbenzyl-\(\gamma\)-CD diols de-\(O\)-benzylated at \(C_6\),\(C_6\) and \(C_6\),\(C_6\) positions, respectively, which could be separated by combined column and HPLC chromatographies. Thus, the treatment of perbenzyl-\(\gamma\)-CD \(1\) with 3 M solution of DIBAL-H in toluene for 41 hours at 0 °C gave a mixture of two isomeric diols \(2a\) and \(2b\), which could be resolved by TLC on silica in a dichloromethane–acetone mixture. On a preparative scale, the symmetrical isomer \(2a\) was partly separated from the mixture by column chromatography on silica using a mixture of dichloromethane–acetone \(98:2\), which allowed isolation of \(2a\) in 14% yield. The residual mixture was separated by preparative HPLC using the same solvent system allowing another crop of \(2a\) (10% yield) and \(2b\) (10% yield). In total, pure compounds \(2a\) and \(2b\) were isolated in 24% and 10% yields, respectively. The identification of the constitution of the isomers was then achieved by NMR methods; compound \(2a\) possesses \(C_2\) symmetry axis, hence \(^1H\) NMR spectrum reveals four signals of H-1 protons (Fig. 1a), whilst the \(C_1\)-symmetrical isomer \(2b\) shows eight doublets, one for each H-1 proton (Fig. 1b). The \(C_6\)-\(C_6\) substitution pattern in \(C_1\)-symmetrical isomer \(2b\), which was proposed with help of “hex-5-enzyme method” in the first report \(^{32}\) on selective de-\(O\)-benzylolation of CDs, is consistent with the full NMR assignment achieved in this work. For further transformations, only the isomer \(2a\) was used. Conversion of the free hydroxyl groups of \(2a\) to bromides was achieved by the action of triphenylphosphane and tetraacetone \(^9\) with potassium thioacetate in DMF at room temperature for 4 hours at 0.8 mM concentration of the starting thioacetate \(^5\) and it was deprotected \(in\) \(situ\) by treatment with aqueous solution of sodium hydroxide (0.17 M) under an inert atmosphere after which the solution was diluted to the required concentration. The pH of the solution was adjusted to \(-9\) by the introduction of gaseous carbon dioxide and the mixture was exposed to air. Monitoring of the oxidation reaction by reversed phase TLC revealed the formation of one product accompanied – above approximately one millimolar concentration – with another material, presumably oligomeric/polymeric by-products, which showed no mobility on an RPTLC plate. The analysis of the major product isolated by reversed phase chromatography revealed its dimeric structure. The structure was confirmed by MALDI–HRMS, NMR and X-ray analysis of a single crystal. In contrast to our previous studies carried out with smaller \(\alpha\)-CD or \(\beta\)-CD homologues the presence of a monomeric product (intramolecular disulfide) was not detected, or this by-product remained hidden with inseparable polymeric material. The preparative run was carried out at 0.8 mM concentration of the starting thioacetate. The product precipitated out of the solution upon neutralization and could be isolated by simple centrifugation in 82% yield. The isolation was possible due to the relatively low solubility of the product – approx. 0.1 mM in water or in a phosphate buffer at pH 7.

The above described procedure allowed the isolation of both \(C_6\),\(C_6\) and \(C_6\),\(C_6\) debenzylated isomers \(2a\) and \(2b\), the former being then converted to the required \(C_2\)-symmetrical dithiol \(6\). In addition to this strategy for the synthesis of duplex \(7\), we have explored two alternative pathways avoiding somewhat laborious separation of the isomers \(2a\) and \(2b\). The first approach relies on the use of the mixture of isomers \(2a\) and \(2b\) for the subsequent two synthetic steps and separation of isomeric \(6\),\(6\)- and \(6\),\(6\)-dibromo-\(\gamma\)-CDs by means of reversed-phase chromatography. Alternatively, the whole synthesis was carried out with the unseparated mixture of \(6\),\(6\) and \(6\),\(6\)-derivatives which gave rise to a mixture of duplexes from which compound \(7\) was isolated by reversed phase chromatography. The separation of the mixture of the three other possible non-symmetrical duplexes was not successful. Both approaches allowed somewhat a more efficient separation of the isomeric derivatives at later stages of synthesis than that of diols \(2a\) and \(2b\). On the other hand, the use of a mixture of isomers precludes an appropriate analysis and characterization of the intermediate compounds (especially by NMR methods) in the course of the synthesis. For this reason, these alternative approaches are not described in detail here, nevertheless the individual synthetic steps are analogous to those described for the transformation of a pure isomer \(2a\).

---

Fig. 1 Part of \(^1H\) NMR (600 MHz) spectra showing H-1 protons of: (a) compound \(2a\), (b) compound \(2b\). Non-labeled signals belong to benzylc protons.
Surfnet algorithm to be 740 Å³ (Fig. 3). Volumes of the analogous distance between O-3 to O-3 located on the opposite rims of the duplex is 12.1 Å, an analogous distance being explained by cis fusion of A and B rings of the former which is apparently more favorable for inclusion into the β-CD macrocycle than α-CD macrocycle (Fig. 2) that exhibits significantly higher stability than that with 5α-cholanic acid (~20 M⁻¹) the difference being explained by cis fusion of A and B rings of the former which is apparently more favorable for inclusion into the β-CD macrocycle than trans AB fused 5α-cholanic acid. In our series all bile acids are cis AB fused and thus other structural features must affect the observed behavior. Interestingly, lithocholic acid (8) appears to be the most hydrophobic com-

Crystal structure

A single crystal suitable for X-ray analysis was grown by the hanging drop method from aqueous dimethyl sulfoxide solutions. Its analyses revealed that the geometry of the parent γ-CD is preserved in the duplex, nevertheless the macrocycles, especially their wider rims, are somewhat elliptically distorted. The lengths of the longer and shorter axes of the pseudoellipse measured between the most proximal opposite C-2 atoms ("a" and "b" in Fig. 2) are 15.3 Å and 12.2 Å, respectively. The length of the duplex expressed as the average internuclear distance ("c" in Fig. 2) between the closest H-3 and H-3' protons located on the opposite rims of the duplex is 12.1 Å, an analogous distance between O-3 to O-3' is about 13.5 Å.

The solvent accessible volume of the inner cavity of duplex 7 in the crystal structure was computed using the Surfnet algorithm to be 740 Å³ (Fig. 3). Volumes of the smaller β- and α-CD homologues reported earlier by us were also calculated and compared with that of duplex 7. Two disulfide bond-connected β- and α-CD duplexes reveal cavities with volumes of 426 Å³ and 296 Å³, respectively. Interestingly, three disulfide bonds in triply-connected α-CD duplex close the cavity in the center of the duplex forming two smaller cavities with volumes of 114 Å³.

Inclusion complexes

The above discussed size parameters of the cavity of duplex 7 indicate that it should be able to efficiently bind larger compounds than its smaller α-CD or β-CD homologues. Thus, the ability to form inclusion complexes was investigated by means of isothermal titration calorimetry in aqueous phosphate buffer at pH 7 using a series of twelve guest compounds that had been used in our previous studies on analogous β-CD duplexes. Out of that series of compounds, a satisfactory fit to 1 : 1 model could only be obtained for deoxycholic acid (9) and hexadecafluorodecane-1,10-dioic acid (12). For other compounds, complexes with more complicated stoichiometry were apparent, for which we were unable to find models allowing a convincing fit. Therefore, we expanded the series of investigated structurally analogous salts of bile acids (Fig. 4) to see whether subtle structural changes translate into apparent binding affinities. These were found to be comparable across the series, being in the range of 5.3 × 10⁷ M⁻¹ – 1.9 × 10⁹ M⁻¹. Although the differences in binding affinities expressed as free energy changes are quite small (0.8 kcal mol⁻¹ between the largest and smallest value), there is a clear distinction between lithocholic acid (8) and the remaining bile acids when comparing their thermodynamic parameters; the complex of 7 with lithocholic acid (8) is strongly enthalpy driven (~11 kcal mol⁻¹) whilst complexes with the other bile acids, in particular with deoxycholic acid (9) and chenodeoxycholic acid (10), show significantly larger entropic components. Lithocholic acid (8) is known to form a strong complex with native β-CD (~20 M⁻¹) that exhibits significantly higher stability than that with 5α-cholanic acid (~20 M⁻¹), the difference being explained by cis fusion of A and B rings of the former which is apparently more favorable for inclusion into the β-CD macrocycle than trans AB fused 5α-cholanic acid. In our series all bile acids are cis AB fused and thus other structural features must affect the observed behavior. Interestingly, lithocholic acid (8) appears to be the most hydrophobic com-

Table 1: Binding affinities and thermodynamic parameters of formation of inclusion complexes of duplex 7 and guest compounds 8–13 (Fig. 5) as determined with isothermal titration calorimetry

| Entry | Guest compound          | Binding stoichiometry (ligand : ?) | $K \pm \sigma_K$ (M⁻¹) | $\Delta H^° \pm \sigma_{H1}$ (kcal mol⁻¹) | $T \Delta S^° \pm \sigma T \Delta S$ (kcal mol⁻¹) |
|-------|-------------------------|-----------------------------------|------------------------|-------------------------------------------|-----------------------------------------------|
| 1     | Lithocholic acid (8)    | 1 : 1                             | 1.90 ± 0.43 × 10⁸     | -11.02 ± 0.08                             | 0.27 ± 0.19                                   |
| 2     | Deoxycholic acid (9)    | 1 : 1                             | 5.31 ± 2.16 × 10⁸     | -3.57 ± 0.06                              | 6.97 ± 0.27                                   |
| 3     | Chenodeoxycholic acid (10)| 1 : 1                             | 1.05 ± 0.60 × 10⁸     | -5.08 ± 0.08                              | 5.86 ± 0.38                                   |
| 4     | Dehydrocholic acid (11) | 1 : 1                             | 8.06 ± 1.83 × 10⁸     | -6.76 ± 0.05                              | 4.03 ± 0.16                                   |
| 5     | Hexadecafluorodecane-1,10-dioic acid (12)| 1 : 1                       | 3.25 ± 0.43 × 10⁸     | -2.37 ± 0.06                              | 5.15 ± 0.13                                   |
| 6     | 6(p-Toluidino)-2-naphthalensulfonic acid (13)| 2 : 1                       | 5.78 ± 0.79 × 10⁸     | -13.62 ± 0.15                             | -4.38 ± 0.13                                  |
| 7     | Deoxycholic acid (9)    | 1 : 1                             | 1.06 ± 1.50 × 10⁷     | -10.91 ± 0.13                             | -1.32 ± 0.83                                  |
Bond (7) duplex the thermodynamic signature of the binding process. The order is observed, as can be deduced from this and previous31 dimers.12,13 It should be noted that within the commonly used contrast to complexes with more flexible singly-bridged the steroid skeleton occupies both cavities of the duplex, in components revealed a probable mode of inclusion (Fig. 5); being oriented inside the – cavity – being oriented outside the cavity to aqueous media. The that each molecule occupies one cavity with the sulfonic group freedom of both guest molecules is restricted. It is assumed that each molecule occupies one cavity with the sulfonic group being oriented outside the cavity to aqueous media. The phenyl rings are likely to overlap in part inside the cavity interacting through π–π stacking as deduced from known complexes of planar aromatic compounds in native γ-CD.38

Conclusions

The synthetic procedures described in this paper allow preparation of both C6, C6 IV and C6, C6 IV bis-O-debenzylated γ-CD isomers. The latter, being symmetrical, was subsequently converted to dithiol and used for the synthesis of the γ-CD duplex connected with two disulfide bonds. The oxidative dimerization proceeded well in 0.1–1 mM concentration range at pH 9 affording γ-CD duplex in high yield. In contrast to our preceding studies on α- or β-CD homologues the formation of a monomeric product was not observed, probably due to un-
favorable relatively large deformation of γ-CD macrocycles required for intramolecular disulfide bond formation.

Due to the large solvent accessible volume of the cavity of the γ-CD duplex (740 Å³), the molecule is ideally suited for complexation of relatively large organic molecules such as steroids. Bile acids examined in this study show binding affinities in the range of 5.3 × 10⁻⁷ M⁻¹–1.9 × 10⁻⁸ M⁻¹, i.e. two to three orders of magnitude larger than the native β-CD which has been known to be the best host for steroid structures among native CDs. The ITC titrations revealed that the cavity of γ-CD duplex is able to accommodate multiple smaller molecules or to form higher aggregates with them. Altogether with smaller α-CD or β-CD homologues we have developed a system of host molecules that enables complexation of the broad range of organic molecules in aqueous media with high binding affinities (Kₐ ~ 10⁻⁷–10⁻⁹ M⁻¹). We presume that they could be used in various indicator displacement assays at low analyte concentrations or for drug delivery purposes.

Acknowledgements

This work was financially supported by the Institute of Organic Chemistry and Biochemistry AS CR v.v.i. (Z40550506), COST (Action CM1005) and by Ministry of Education, Youth and Sport of the Czech Republic (LD12019). M. D. is indebted to the Czech Science Foundation for the support of the project 14-03276S.

References

1. H. E. Dodziuk, *Cyclodextrins and Their Complexes*, Wiley-VCH, Weinheim, 2006.
2. L. Liu and Q.-X. Guo, *J. Inclusion Phenom. Macrocyclic Chem.*, 2002, 42, 1–14.
3. K. N. Houk, A. G. Leach, S. P. Kim and X. Zhang, *Angew. Chem., Int. Ed.*, 2003, 42, 4872–4897.
4. T. Kraus, *Curr. Org. Chem.*, 2011, 15, 802–814.
5. F. Bellia, D. La Mendola, C. Pedone, E. Rizzarelli, M. Saviano and G. Vecchio, *Chem. Soc. Rev.*, 2009, 38, 2756–2781.
6. M. V. Rekharsky and Y. Inoue, *Chem. Rev.*, 1998, 98, 1875–1917.
7. E. V. Anslyn, *J. Org. Chem.*, 2007, 72, 687–699.
8. B. T. Nguyen and E. V. Anslyn, *Coord. Chem. Rev.*, 2006, 250, 3118–3127.
9. W. M. Nau, G. Ghale, A. Hennig, H. s. Bakirci and D. M. Bailey, *J. Am. Chem. Soc.*, 2009, 131, 11558–11570.
10. G. Ghale, V. Ramalingam, A. R. Urbach and W. M. Nau, *J. Am. Chem. Soc.*, 2011, 133, 7528–7535.
11. Y. Liu and Y. Chen, *Acc. Chem. Res.*, 2006, 39, 681–691.
12. Y. Liu, Y. W. Yang, E. C. Yang and X. D. Guan, *J. Org. Chem.*, 2004, 69, 6590–6602.
13. P. R. Cabrer, E. Azvarez-Parrilla, F. Meijide, J. A. Seijas, E. R. Nunez and J. V. Tato, *Langmuir*, 1999, 15, 5489–5495.
14. I. Tabushi, Y. Kuroda and K. Shimokawa, *J. Am. Chem. Soc.*, 1979, 101, 1614–1615.
15. O. Bistri, K. Mazeau, R. Auzely-Velty and M. Sollogoub, *Chem. – Eur. J.*, 2007, 13, 8847–8857.
16. R. Breslow and S. Chung, *J. Am. Chem. Soc.*, 1990, 112, 9659–9660.
17. D. Q. Yuan, S. Immel, K. Koga, M. Yamaguchi and K. Fujita, *Chem. – Eur. J.*, 2003, 9, 3501–3506.
18. D.-Q. Yuan, K. Koga, I. Kouno, T. Fujikoa, M. Fukudome and K. Fujita, *Chem. Commun.*, 2007, 828–830.
19. T. Lecourt, J. M. Mallet and P. Sinay, *Tetrahedron Lett.*, 2002, 43, 5533–5536.
20. T. Lecourt, J. M. Mallet and P. Sinay, *Eur. J. Org. Chem.*, 2003, 4553–4560.
21. O. Bistri, T. Lecourt, J. M. Mallet, M. Sollogoub and P. Sinay, *Chem. Biodiversity*, 2004, 1, 129–137.
22. T. Lecourt, P. Sinay, C. Chassenieux, M. Rinaudo and R. Auzely-Velty, *Macromolecules*, 2004, 37, 4635–4642.
23. D. X. Dong, D. Baigl, Y. L. Cui, P. Sinay, M. Sollogoub and Y. M. Zhang, *Tetrahedron*, 2007, 63, 2973–2977.
24. O. Bistri-Aslanoff, Y. Bleriot, R. Auzely-Velty and M. Sollogoub, *Org. Biomol. Chem.*, 2010, 8, 3437–3443.
25. L. Kumprecht, M. Buděšínský, J. Vondrásěk, J. Vymětal, J. Černý, I. Cisařová, J. Brynda, V. Herzig, P. Koutník, J. Závada and T. Kraus, *J. Org. Chem.*, 2009, 74, 1082–1092.
26. L. Krejčí, M. Buděšínský, I. Cisařová and T. Kraus, *Chem. Commun.*, 2009, 3557–3559.
27. A. Wang, W. Li, P. Zhang and C.-C. Ling, *Org. Lett.*, 2011, 13, 3572–3575.
28. R. Breslow, N. Greenspoon, T. Guo and R. Zarzycki, *J. Am. Chem. Soc.*, 1989, 111, 8296–8297.
29. L. Kumprecht, M. Buděšínský, P. Bouř and T. Kraus, *New J. Chem.*, 2010, 34, 2254–2260.
30. P. Maloň, L. Bednarová, M. Straka, L. Krejčí, L. Kumprecht, T. Kraus, M. Kubanová and V. Baumruk, *Chirality*, 2010, 22, E47–E55.
31. A. Grishina, S. Stanchew, L. Kumprecht, M. Buděšínský, M. Pojarová, M. Dušek, M. Rumlová, I. Křižová, L. Rulišek and T. Kraus, *Chem. – Eur. J.*, 2012, 18, 12292–12304.
32. A. J. Pearce and P. Sinay, *Angew. Chem., Int. Ed.*, 2000, 39, 3610–3612.
33. T. Lecourt, A. Herault, A. J. Pearce, M. Sollogoub and P. Sinay, *Chem. – Eur. J.*, 2004, 10, 2960–2971.
34. R. A. Laskowski, *J. Mol. Graphics*, 1995, 13, 323–330.
35. Due to pKₐ ~ 5 of the investigated bile acids in aqueous solutions they are nearly completely dissociated under the conditions (pH 7) of ITC experiments. For reference see: A. Fini and A. Roda, *J. Lipid Res.*, 1987, 28, 755–759.
36. Z. W. Yang and R. Breslow, *Tetrahedron Lett.*, 1997, 38, 6171–6172.
37. O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, 31, 455–461.
38. A. Nakamura and Y. Inoue, *J. Am. Chem. Soc.*, 2002, 125, 966–972.