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Synthesis and binding properties of cyclodextrin trimers

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Abstract—A series of cyclodextrin trimers and dimers were prepared and examined as binders for appropriate trimeric and dimeric aminoacid amides. Tritopic binding was stronger than ditopic binding, although the free energies were not strictly additive. Such trimers are attractive prospects for the binding of polypeptides and proteins. © 2001 Elsevier Science Ltd. All rights reserved.

We have been interested in sequence–selective binding of peptide sidechains in water using various cyclodextrin (CD) derivatives. Although individual CDs bind these amino acid residues very weakly ($k \leq 200$ M^{-1}),[1] we have found that CD dimers selectively bind to certain peptides[2] and proteins[3] in aqueous solution and with much higher affinity compared to β-CD. Introduction of a third binding cavity ought to provide an even higher binding affinity and selectivity. However, few CD trimers have been reported to date,[4] and even less is known about the binding properties of these CD trimers. We thus, synthesized new CD trimers and studied the binding characteristics of these novel compounds.

Synthesis of CD trimers is more difficult than for the analogous CD dimers. Reactions tend to be low yielding, in part due to steric hindrance. This is especially true for centrally-linked CD trimers that have a small core; once two CDs have been attached, the third one can be less reactive. Luo and coworkers[4] reported incomplete alkylation, resulting in a mixture of CD dimers and trimers. We reason that a simple and efficient way to prepare CD trimers would employ amide chemistry, in which the coupling reactions are high yielding, specific, and predictable. Using pentafluorophenyl ester as the activated acyl group, we were able to synthesize a variety of centrally- and linearly-linked CD trimers **1**–**4** in up to 80% yield with no detectable dimer formation.

Trimer **1** was prepared in 80% yield by acylating 6-amino-6-deoxycycloheptaamylose[5] with the tris(pentafluorophenyl) ester of trimesic acid in DMF with triethylamine, reacting overnight at ambient temperature. Then the product was isolated by reverse phase chromatography on a LOBAR column with a methanol/water gradient, and the solvent was removed by lyophilization. Trimers **2** and **3** were prepared in a similar manner with the tris(pentafluorophenyl) esters of tris(4-carboxyphenyl)amine and nitrilotriacetic acid, respectively, in DMF with triethylamine. The bisfunctionalized central CD of trimer **4** was synthesized by acylating A,D-bis-(6-amino-6-deoxy)cycloheptaamylose[6] with 2 equiv. of 1-methyl-4-pentafluorophenylterephthalate in DMF with diisopropylethylamine. Isolation of the bis-amide in 65% yield was again by reverse phase gradient chromatography. Deprotection and then acylation with 2 equiv. of 6-amino-6-deoxycycloheptaamylose in DMF with PyBop yielded trimer **4** in 35% overall yield. We also made CD dimers **5** and **6** for control purposes. They were prepared by acylating 6-amino-6-deoxycycloheptaamylose with terephthalic acid bis-pentafluorophenyl ester and isophthalic acid bis-pentafluorophenyl ester, respectively, in DMF with diisopropylethylamine. All dimers and trimers were white solids, and all were characterized by H NMR and MALDI-TOF mass spectrometry. For example, trimer **1** had MS (MALDI-TOF) of 3581.7 ($M+Na^+$) and trimer **4** had MS (MALDI-TOF) of 3684.0 ($M+Na^+$).

Employing the same general coupling methodology, we prepared a series of substrates. Substrates **a** was prepared by acylating tryptophan methyl ester with the tris(pentafluorophenyl) ester of trimesic acid, followed by saponification and acidification to yield the free acid. Substrates **b** and **c** were prepared by acylating 4-*t*-butylphenylalanine with the pentafluorophenyl esters of trimesic acid and isophthalic acid, respectively, in DMF–CH_2Cl_2. Substrate **d** was prepared by acylating tryptophan methyl ester with the acyl chloride

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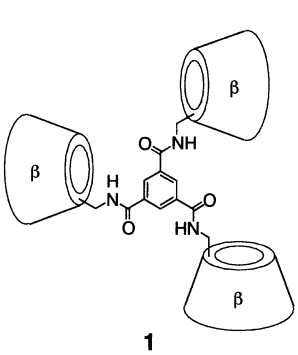

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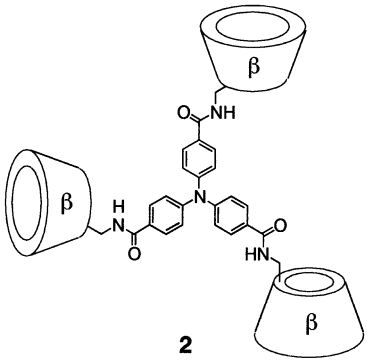

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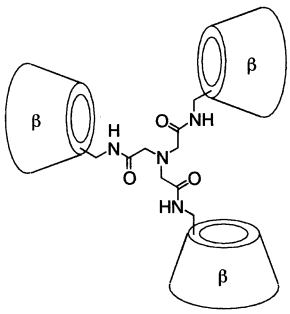

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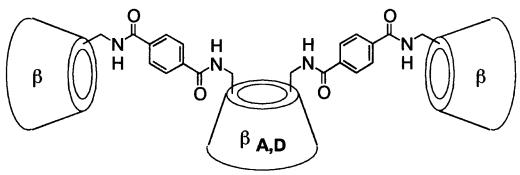

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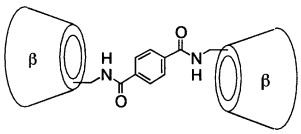

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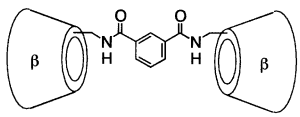

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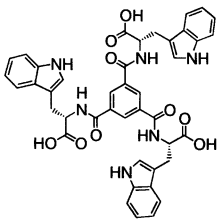

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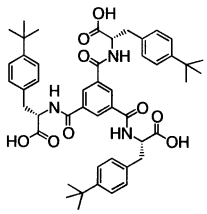

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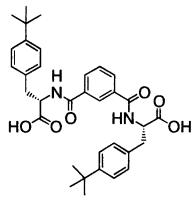

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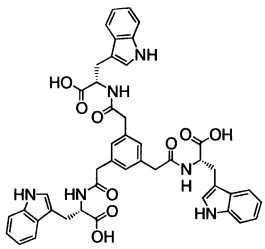

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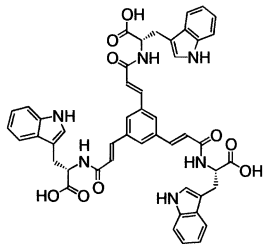

e

of 1,3,5-benzene-triacetic acid[7] followed by saponification and acidification. Substrate **e** was prepared from reacting the acyl chloride of 1,3,5-bezenetriacrylic acid[8] with tryptophan methyl ester, followed by saponification and acidification**.** All of the substrates were characterized by H NMR and APCI+ mass spectrometry.

Binding studies were performed either by titration microcalorimetry or by NMR titration. All titration microcalorimetry studies were conducted in 0.2 M pH 9.0 $NaHCO_3/Na_2CO_3$ buffer in water at 25°C. Substrate solutions (typically 3 mM in a 250 μL syringe) were titrated into a solution of CD hosts (typically 0.3

mM in the cell). In a typical NMR titration experiment, the substrate was dissolved to a final concentration of 1–3 mM in 0.2 M pH 9.0 $NaHCO_3/Na_2CO_3$ buffer in water at 25°C with a 1 μL of acetonitrile added as internal standard. In a series of experiments a given CD trimer was added as solid in portions typically from 0.5 to 5 equiv. Spectra were collected on a 400 MHz Bruker spectrometer employing a water suppression protocol. Changes in chemical shifts (ΔHz) in the aromatic protons were measured, and plotted against CD trimer concentrations. The data was then fitted with a non-linear least-squares regression program to estimate the binding constant, assuming a 1:1 complex.[9,10]

Our initial intent was to focus on binding studies with substrates (**a, d,** and **e**) that incorporate the natural amino acid tryptophan. However, as Table 1 shows, the binding constants of trimer **1** binding to substrates **a** and **d** are only slightly higher than that of β-CD to tryptophan ($\Delta G = -13.3$ kJ/mol)[1] but not as good as expected for multimeric binding. Therefore, we modified the binding groups on the substrates to *tert*-butylphenylalanine (*t*BuPhe), which has a high binding affinity to CD, to gain insight into the structural features that are required to achieve strong 1:1 binding. Binding constants K_1 to K_3 from titrating substrate **b** to β-CD are similar to each other and comparable to that of *t*BuPhe to β-CD ($\Delta G = -21.6$ kJ/mol). Thus, the three *t*Bu-phenyl groups in substrate **b** could accommodate binding of three CDs. Incorporation of the second CD cavity in dimer **6** lowered the energy of the complex by 12 kJ/mol compared to the monomeric binding, and the introduction of the third CD further lowered it by another 3 kJ/mol. A similar trend is seen in comparing **4**-**b** and **5**-**c** complexes.

Interestingly, binding of **c** to **1** is weaker than **c** to **6**. The presence of the third CD in trimer **1** actually destabilizes the complex by 5.3 kJ/mol., presumably by restricting the other two CDs to a less favorable geometry for binding. However, this unfavorable interaction is more than compensated by the introduction of a third *t*Bu-phenyl group in the substrate. As Table 1 shows, binding of **1** with **b** lowers the energy of the complex by 8.5 kJ/mol. compared to binding of **1** to **c**. Thus, the third CD indeed participates in the binding of the trimeric substrate and stabilizes the trimeric complex.

Table 1. Binding constants[a]

Host	Guest	Host:guest	K_a (M^{-1})	$\Delta G°$ (kJ/mol)
1	**a**		No detectible binding	N/A
1	**a***	1:1	650 ± 90	-16.1 ± 0.3
1	**b**	1:1	$3.5 \pm 0.6 \times 10^6$	-37.3 ± 0.4
1	**c**	1:1	$1.1 \pm 0.2 \times 10^5$	-28.8 ± 0.4
1	**d***	1:1	280 ± 60	-14.0 ± 0.5
1	**e***		No detectible binding	N/A
2	**b**	1:1	$6.1 \pm 0.5 \times 10^5$	-33.0 ± 0.2
3	**b**	1:1	$7.4 \pm 0.8 \times 10^5$	-33.5 ± 0.3
4	**b**	1:1	$4.1 \pm 0.4 \times 10^6$	-37.7 ± 0.2
5	**c**	1:1	$8 \pm 2 \times 10^5$	-33.7 ± 0.6
6	**c**	1:1	$9 \pm 1 \times 10^5$	-34.1 ± 0.3
β-CD	*t*BuPhe	1:1	$6 \pm 1 \times 10^3$	-21.6 ± 0.4
β-CD	**b**		$6.0 \pm 0.9 \times 10^3$ (K_1)	-21.6 ± 0.4
			$5.5 \pm 0.8 \times 10^3$ (K_2)	-21.3 ± 0.4
			$6.8 \pm 0.9 \times 10^3$ (K_3)	-21.9 ± 0.3

[a] Determined by microcalorimetric titration except noted by *, which was done by NMR titration.

In binding substrate **b** to trimers **1** to **4**, no higher complexes were detected, as evident by the lack of heat release in microcalorimetry titration with additional equivalents of guest molecules. Of the four trimers, **2** and **3** show lower binding affinities to **b** compared to **1** and **4** but comparable affinities to the dimeric bindings of **5** and **6** to **c**. The lower binding affinity could be due to mismatch in linker lengths. Alternatively, perhaps only two CDs are used to bind these substrates. However, we did not observe formation of higher order complexes; thus even with their diminished binding affinity, **2** and **3** still prefer to bind one guest over two. Hence, the different binding properties of different trimers are likely to be a consequence of the matching of host–guest geometry. Interestingly, trimer **4** shows as high an affinity toward **b** as trimer **1**. Whereas a 1:1 complexation in the case of trimer **1** would require all-primary or all-secondary face binding, the three CD's in trimer **4** in principle can bind the substrate from different faces. We do not know which face(s) is responsible for the binding, but the formation of a 1:1 complex with trimer **4** and **b** suggests that **4** can curl up to better fit the substrate binding groups.

Tritopic binding in this series does indeed lead to strong selective complexing, but with less than full additivity of the free energies. Simple full additivity would have led to a binding free energy of −64.8 kJ/mol. These and related CD trimers are thus good candidates for even more effective binding to polypeptides and proteins than the CD dimers we reported previously.[2,3]

Acknowledgements

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References

1. Rekharsky, M.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875–1917.
2. Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536–3537.

3. Leung, D.; Yang, Z.; Breslow, R. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5050–5053.
4. Luo, M.; Chen, W.; Yuan, D.; Xie, R. *Synth. Commun.* **1998**, *28*, 3845–3848.
5. (a) Hanessian, S.; Benalil, A.; Laferriere, C. *J. Org. Chem.* **1995**, *60*, 4786–4797; (b) Hanessian, S.; Benalil, A.; Viet, M. T. *Tetrahedron* **1995**, *51*, 10131–10148.
6. Khan, A.; Forgo, P.; Stine, K.; D'Souza, V. *Chem. Rev.* **1998**, *98*, 1977–1996.
7. Cochrane, W.; Pauson, R.; Stevens, T. *J. Chem. Soc. C* **1968**, 630–632.
8. Effenberger, F.; Kurtz, W. *Chem. Ber.* **1973**, *106*, 511–524.
9. Guo, Q. *J. Incl. Phenom.* **1994**, *17*, 149.
10. Connors, K. *Binding Constants*: *The Measurement of Molecular Complex Stability*, 1st ed.; John Wiley & Sons: New York, 1987.