

Sequence Selective Binding of Peptides by Artificial Receptors in Aqueous Solution

Ronald Breslow,* Zhiwei Yang, Rachel Ching,
Gunter Trojandt, and Fabrice Odobel

Department of Chemistry, Columbia University
New York, New York 10027

Received November 24, 1997

We have been interested in preparing cyclodextrin dimers that could bind peptide side chains in water with sequence selectivity.¹ To explore this, we have made dimers of β -cyclodextrin **1–6** and examined their binding to peptides **a–e** and to the dipeptides Phe-Phe and Trp-Trp.³ All binding studies (except with **6**) were performed by titration microcalorimetry,^{1,4} adding the peptide solutions to a solution of the dimer host in 0.2 M pH 9.0 NaHCO₃/Na₂CO₃ buffer in water at 25 °C. The results, listed in Table 1, indicate in K_1 the binding constant for formation of a 1:1 complex, while K_2 is the constant for binding of a second peptide to the 1:1 complex (except in the case of simple β -cyclodextrin, which was added to a solution of peptide **a**, so K_2 corresponds to binding of a second cyclodextrin to the peptide).

As the data in Table 1 show, dimer **6** with a short disulfide link on the primary cyclodextrin face binds well to the simple dipeptide Trp-Trp. The strength of binding exceeds that for simple cyclodextrin¹ or for dimers **1**, **2**, or **5** (Table 1), indicating that **6** specifically recognizes and binds both Trp side chains. By contrast, dimer **5** with a longer link on the cyclodextrin primary face⁵ binds weakly to Trp-Trp. It also binds more weakly to peptide **a** than does simple β -cyclodextrin. The binding by the two cyclodextrin units of **5** to Trp-Trp or to **a** is not cooperative. Chelate binding of two linked cyclodextrins to a peptide with two hydrophobic side chains would lead to a large K_1 and a small K_2 , but K_1 is not larger than K_2 for **5** either with peptide **a** or with Trp-Trp. The binding of **a** with simple β -cyclodextrin has values of K_1 and of K_2 (Table 1) that are similar to the 180 M⁻¹ determined previously for binding of β -cyclodextrin to monomeric L-Phe-D-Pro.⁶ The problem in the binding of **5** to **a** comes from putting the link on the primary faces of the cyclodextrins. It was reported previously⁶ that β -cyclodextrin binds well to peptides carrying the sequence L-Phe-D-Pro (or D-Phe-L-Pro), and it was proposed—based on NMR and computational evidence—that binding was into the secondary face of the cyclodextrin, with a segment of the Pro inserting into the wide secondary face along with the phenyl unit of Phe. Thus we have examined cyclodextrin

(1) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1992**, *114*, 5882–5883. Reexamination of the binding of Trp-Trp to a bipyridyl-linked cyclodextrin dimer described in that communication indicates a K of only 40 M⁻¹, much smaller than first reported and indicating no chelate binding with this long linker. The binding of Trp-Trp is accompanied by a fluorescence increase, which was used for the original study, but our later work indicates that several effects contribute to this change; it is not as reliable for binding constant determination as the NMR technique used in the current work, or the calorimetric method. However, Trp-Trp has a binding constant of 1200 M⁻¹ (see Table 1) to the β -cyclodextrin dimer **6** that we had reported earlier,² so this dimer with a short linker does achieve chelate binding of the peptide. We determined this from the NMR shifts of the phenyl protons of the tryptophan rings, ca. 20 Hz upfield shifts at 500 MHz, on binding into the cyclodextrin cavities.

(2) Breslow, R.; Greenspoon, N.; Guo, T.; Zarzycki, R. *J. Am. Chem. Soc.* **1989**, *111*, 8296–8297.

(3) The synthesis and characterization of all new compounds is described in the Supporting Information.

(4) Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1993**, *115*, 9353–9354.

(5) On C-6 of the cyclodextrin, by alkylation of the dithiol with the cyclodextrin-6-iodide.

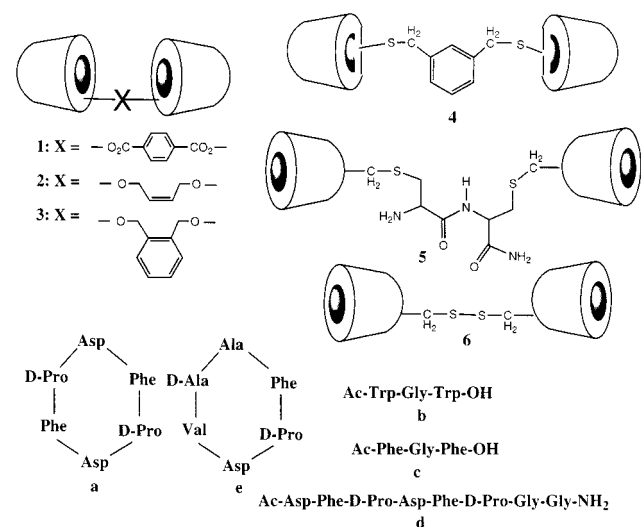
(6) Maletic, M.; Wennemers, H.; McDonald, D. Q.; Breslow, R.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1490–1492.

Table 1. Binding Constants^a

host	peptide	K_1 (M ⁻¹) ^b	K_2 (M ⁻¹) ^c
1	a	2590 ± 90	1120 ± 30
1	b	130 ± 10	117 ± 6
1	c	91 ± 6	85 ± 6
1	d	1100 ± 200	114 ± 24
1	e	930 ± 35	452 ± 17
1	Trp-Trp	84 ± 2	93 ± 2
2	a	675 ± 32	97 ± 5
2	Trp-Trp	87 ± 3	86 ± 3
2	Phe-Phe	123 ± 4	49 ± 2
3	a	590 ± 25	100 ± 6
4	a, b, c	no detectable binding	
5	a	98 ± 4	105 ± 5
5	Trp-Trp	96 ± 2	98 ± 3
β -cyclodextrin	a	220 ± 18	300 ± 27
6	Trp-Trp	1200 ± 400 ^d	

^a Determined by microcalorimetric titration in 0.2 M pH 9.0 NaHCO₃/Na₂CO₃ buffer at 25 °C. ^b Constant for formation of a 1:1 complex between host and peptide. ^c Constant for binding of a second molecule of peptide to the 1:1 complex, except for the cyclodextrin entry where it is a second molecule of cyclodextrin binding to the complex. ^d By NMR titration in D₂O; the large uncertainty arises because of some self-association of the peptide at high concentrations.

Chart 1



dimers linked on the secondary face, to permit stronger cooperative binding into the secondary faces of the two cyclodextrins.

A simple way to link cyclodextrins on their secondary face is to react the secondary manno-epoxide with a thiol.⁷ While this procedure can be used to make a dimer,⁸ one of the glucose units of the cyclodextrin undergoes a conformational change, causing an indentation in the cyclodextrin cavity, because of the inversion at C2 and C3.⁷ We prepared dimer **4** by reacting the secondary manno-epoxide with *p*-phenylene dithiol, but it gave no detectable binding with peptides **a**, **b**, or **c**. To prevent such a modification of the cavity, we linked two cyclodextrins on their secondary faces by direct alkylation or acylation of the C2 hydroxyl groups. These are the most acidic hydroxyl groups, and are converted to the oxide anion with NaH.

In dimer **3** this was done by alkylation of β -cyclodextrin with *o*-bis(chloromethyl)benzene and NaH. Ishimaru recently reported

(7) Cf.: Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390–1391.

(8) Jiang, T.; Sukumaran, D. K.; Soni, S.-D.; Lawrence, D. S. *J. Org. Chem.* **1994**, *59*, 5149–5155.

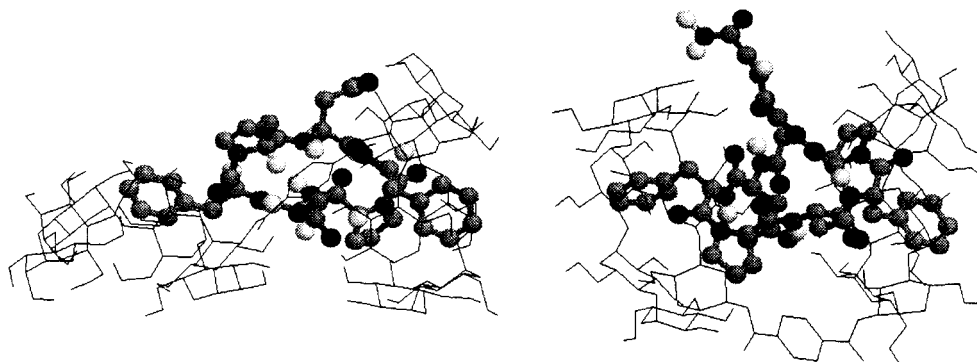


Figure 1. Calculated minimum energy conformations of the complexes of dimer **1** with cyclic peptide **a** (left) and with linear analogue **d** (right). Macromodel was used with water solvation included. The dimer is shown in linear representation, the peptides as ball-and-stick with hydrogens white but deleted on carbons and oxygens in black. In the cyclic case the L-Phe and D-Pro rings are fully bound into one cyclodextrin of **1**, but only the L-Phe into the other cyclodextrin. With the more flexible linear peptide both L-Phe-D-Pro sequences can be fully bound, leading to a larger chelate effect.

an analogous reaction with *m*-bis(chloromethyl)benzene, and also showed that the linkage was at the more reactive C2 positions.⁹ In dimer **2** we linked the cyclodextrin with *cis*-1,4-dichloro-2-butene. To make dimer **1**, we acylated the β -cyclodextrin anion (NaH) with terephthaloyl dichloride. Acyl migration can scramble positions 2 and 3, so dimer **1** is ambiguously acylated on either oxygen and is probably a mixture.

Dimers **1**, **2**, and **3** show cooperative chelate binding to some, but not all, peptides. Dimer **1** shows a large K_1 with peptides **a** and **d**, which have two L-Phe-D-Pro sequences spaced by Asp (for water solubility), and K_2 is smaller. However, no strong chelate binding is seen with Trp-Trp, with **b** (with a Trp-Gly-Trp sequence), or with **c** (with a Phe-Gly-Phe sequence). Peptide **e** shows some cooperative chelate binding, presumably involving the Phe-D-Pro and the Val-D-Ala, but less strong than with the two Phe-D-Pro segments of **a**.

Dimer **1** binds the cyclic peptide **a** more strongly than the linear analogue **d**, but the chelate effect is stronger with **d** (i.e., the ratio of K_1 to K_2 is larger for **d**). Our computer modeling (Figure 1) indicates that the flexible **d** actually fits better into the two cavities of **1** than does the rigid **a**. We believe that this is correct, but that the absolute value of K_1 is smaller for **d** than for **a** because flexible **d** exists in water with internal hydrophobic binding of the two Phe groups. This must be broken for **d** to bind to **1**, but in rigid **a** no such competitive preorganization has to be disrupted.

Dimer **2** also shows strong chelate binding to peptide **a** compared with peptides Trp-Trp or Phe-Phe, and dimer **3** shows similar data for the binding of **a**. Thus the dimer **1** linked on the secondary face of the cyclodextrins shows good selectivity for

binding peptides with sequences Phe-D-Pro-X-Phe-D-Pro, and to a lesser extent Phe-D-Pro-X-Val-D-Ala, but does not bind

Trp-X-Trp, Phe-X-Phe, or Trp-Trp. Dimers **2** and **3** have similar preferences. By contrast, the dimer **6** with a short linker on the primary face binds Trp-Trp well. The selectivities reflect both the lengths of the linkers and their mode of attachment in the cyclodextrin dimers.

Others have reported binding to peptides^{10,11} by ionic or hydrogen-bonding interactions, but we believe ours are the first examples¹ in which the double binding of hydrophobic side chains is used to chelate a receptor to a peptide. Of course our systems can be extended by adding ionic recognition to the hydrophobic recognition we have demonstrated. It will be interesting to see whether such peptide receptors can be used to inhibit the action of peptide hormones or proteins, or as the basis for sequence selective peptide cleaving reagents.

Acknowledgment. Support of this work by the NIH and the NSF is gratefully acknowledged. Rachel Ching was the recipient of a Pfizer Undergraduate Summer Fellowship in Synthetic Chemistry, Gunter Trojandt was a Humboldt Postdoctoral Fellow, and Fabrice Odobel was a Lavoisier Postdoctoral Fellow.

Supporting Information Available: Synthesis and characterization of new compounds (7 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA973991Y

(10) Wennemers, H.; Still, W. C. *Tetrahedron Lett.* **1994**, *35*, 6413–16 and subsequent work.

(11) Albert, J. S.; Goodman, M. S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1995**, *117*, 1143–1144.

(9) Ishimaru, Y.; Macuda, T.; Iida, T. *Tetrahedron Lett.* **1997**, *38*, 3743–3744.