Peptidomimetic Host That Binds a Peptide Guest Affording a β-Sheet Structure That Subsequently Self-Assembles. A Simple Receptor Mimic

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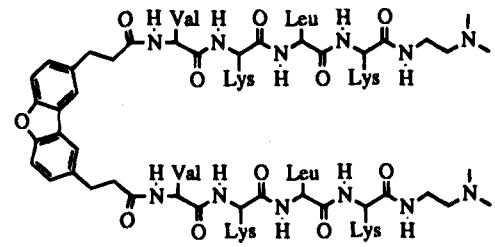
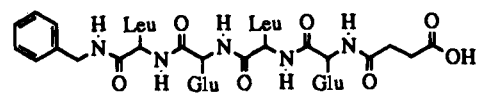
Received September 19, 1994

In an attempt to understand the energetics of β-sheet-based molecular recognition in aqueous solution, we have constructed a peptidomimetic host that binds to a tetrapeptide guest affording a host–guest complex, which subsequently self-associates into a high molecular weight β-sheet. This system, in the simplest sense, mimics the binding and self-association properties exhibited by receptors[1] such as the class 1 major histocompatibility proteins[2] although association and signaling are not features exhibited by this receptor mimic. The host, which is composed in part of alternating cationic and hydrophobic α-amino acid residues, is selective for anionic guests having an amphiphilic periodicity of 2. Preliminary binding data suggest that both hydrophobic and electrostatic interactions are critical for β-sheet-mediated binding. Ongoing systematic studies with this system should complement the synthetic hosts that have been used to better understand the energetics of ligand binding in molecular recognition.[3]

The receptor mimic (**1**) (Figure 1) was synthesized by coupling 2,8-dibenzofurandiylbis(3-propanoic acid) and the side chain protected peptide V-K(ClZ)-L-K(ClZ)-DMDA.[4] Side chain deprotection was accomplished by hydrogenation[5] resulting in a crude receptor mimic **1**, which was purified by C18 RP-HPLC and characterized by MALDI-TOFMS.[6] The dibenzofuran diacid residue was envisioned to separate the two covalently attached peptide strands by approximately 10 Å, allowing a peptide guest to bind between the strands of **1**, affording a three-stranded, antiparallel β-sheet. The peptide guest, Suc-Glu-Leu-Glu-Leu-NH-Bzyl (**2**) (Figure 1) was prepared on oxime resin,[4a] cleaved from the resin utilizing benzylamine, deprotected, purified, and characterized as described for **1**. The C-terminal benzamide group in guest **2** was envisioned to bind to the dibenzofuran moiety of **1** via an aromatic–aromatic interaction[7] initiating the binding of **2** to **1**,





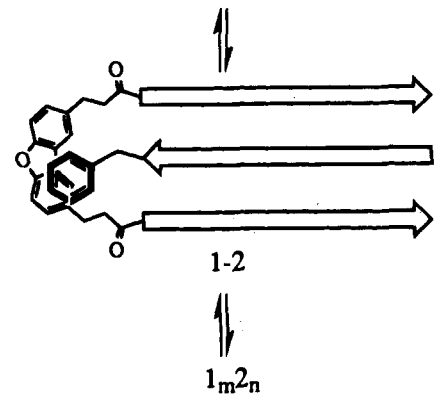


Figure 1. Structure of host (**1**) and guest (**2**) and the apparent binding/association pathway.

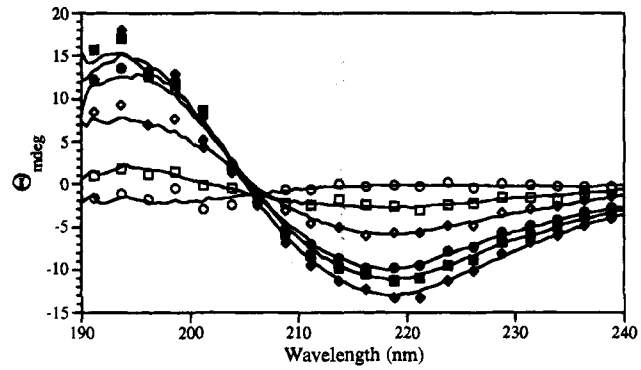


Figure 2. Titration of **1** (50 μM) with **2** in 10 mM NaOAc, pH 5.75. Concentrations of **2**: ○, 0 μM; □, 40 μM; ◇, 80 μM; ●, 120 μM; ■, 160 μM; , ◆, 200 mM.

ultimately affording an intermolecular antiparallel β-sheet composed of **1** and **2**. The peptide sequences in **1** and **2** have an amphiphilic periodicity of 2, allowing the hydrophobic and oppositely charged side chains of **1** and **2** to interact, enhancing sheet stability.[8] When studied separately, **1** and **2** exhibit concentration-independent random coil type circular dichroism (CD) spectra at pH = 5.75, consistent with nonassociating peptides which adopt multiple conformations. The addition of peptide **2** to the receptor mimic **1** affords a CD spectrum with a minimum at 219 nm and a maximum at 194 nm, consistent with a β-sheet complex, Figure 2.[9]

The three-stranded β-sheet complex **1**–**2** resulting from the interaction of peptides **1** and **2** is amphiphilic and as a result

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0002-7863/95/1517-1655$09.00/0

was expected to dimerize in a face to face fashion through hydrophobic interfacial interactions.[8b] The dimerized complex $\mathbf{1}_2\mathbf{2}_2$ may also assemble beyond the dimer by way of intermolecular association of $\mathbf{1}_2\mathbf{2}_2$ complexes, mediated by excess guest peptide **2** into an extended sheet structure. The CD-monitored binding of **1** to **2** could be fitted to the equilibria $\mathbf{1} + \mathbf{2} \rightleftharpoons \mathbf{1}-\mathbf{2} \rightleftharpoons \mathbf{1}_m\mathbf{2}_n$, having a K_D of 490 μM for **1**−**2** and an apparent K_D of 400 nM for $\mathbf{1}_m\mathbf{2}_n$.[10]

In order to better characterize the initial binding reaction of $\mathbf{1} + \mathbf{2} \rightleftharpoons \mathbf{1}-\mathbf{2}$, **1** was covalently attached to an *N*-hydroxysuccinimide activated agarose chromatography resin for high-performance affinity chromatography (HPAC) analysis.[11a] Immobilizing **1** on a resin by an amide bond derived from one of the ϵ-amino groups of lysine ensured that the **1**−**2** complex would not undergo self-association, therefore uncoupling the initial binding equilibrium from the subsequent association equilibria. The dissociation constant for the **1**−**2** complex was determined by HPAC by plotting the concentration of the guest injected versus the reciprocal of the difference between the elution volume and the void volume.[11] The K_D determined by HPAC (510 μM) was virtually identical to that determined by fitting the solution binding data computationally (490 μM).

Analogs of the guest peptide **2**, Suc-ELEL-NH**X**, which differ with regard to the C-terminal amide group (**X**), were evaluated for their ability to bind to the immobilized host using HPAC analysis. As **X** increases in hydrophobicity so does the binding affinity for the host (**X** = H, K_D = 0.76 mM; **X**=isobutyl, K_D = 0.71 mM; **X** = benzyl, K_D = 0.51 mM), but not dramatically. Importantly, **1** is very selective with regard to the tetrapeptides that it is capable of binding. A structural analog of **2** where the glutamic acid residues are replaced with serine has weak affinity (K_D = 2.18 mM), whereas peptides such as Gly_4, Ala_4, and TVTV do not bind to **1**, suggesting that both the amphiphilicity and the anionic nature of the guest are important. An evaluation of the binding energetics of a variety of structural analogs of **2** is currently in progress.

The effect of temperature and salt on the quaternary structure of the **1**−**2** complex was ascertained by evaluating samples containing 50 μM **1** and 410 μM **2**. As the temperature of a sample was increased from 5 to 30 °C, there was little change in the CD spectrum, reflecting the thermal stability of the β-sheet-based $\mathbf{1}_m\mathbf{2}_n$ assembly.[12a-c] Addition of **2** to **1** in the presence of salts differing in their anion component strongly inhibits guest binding as predicted by the electroselectivity series, indicating that the anions bind to **1**. At 25 °C, sulfate prevents the formation of a **1**−**2** complex by inhibiting the binding event at concentrations of 50 mM, while at the same concentrations perchlorate and chloride anions do not appear to significantly perturb the formation of a **1**−**2** complex. At concentrations above 50 mM, perchlorate and chloride inhibit the binding of **1** to **2**.[12b-d]

Since CD spectroscopy is not very sensitive to β-sheet quaternary structure formation,[13] we set out to characterize the MW of the $\mathbf{1}_m\mathbf{2}_n$ complex by analytical equilibrium ultracentrifugation.[14a] These experiments reveal that the $\mathbf{1}_m\mathbf{2}_n$ complex assembled far beyond the dimeric state at 25 °C at pH = 5.75. In fact, the sample sedimented from the equilibrium ultracentrifugation cell at 3000 rpm, dictating that the $\mathbf{1}_m\mathbf{2}_n$ complex has a MW_{app} approaching or exceeding 10^5.[14] The concentrations of both **1** and **2** were varied, as were the temperature, pH, salt type, and salt concentration in an effort to create well-defined quaternary structures composed of **1** and **2** as probed by analytical equilibrium ultracentrifugation. The results of these efforts fall into two categories: either the conditions afforded a soluble **1**−**2** complex of high MW or the conditions denatured the complex, affording unstructured **1** and **2**. These results and others strongly suggest that the binding of **2** by **1** and the subsequent self-association of the **1**−**2** complex are linked equilibria.[10] The self-association has a significantly lower K_D ($K_{D(app)}$ = 400 nM) than the K_D for the formation of **1**−**2** (K_D 490 μM), explaining the cooperative nature of the binding and self-association steps. Strategies pursuant to the formation of a stable $\mathbf{1}_2\mathbf{2}_2$ structure in solution are currently in progress.

Acknowledgment. We would like to acknowledge Dr. Donald Albright and Mr. Joel Schneider for their assistance in performing analytical ultracentrifugation experiments. We would also like to thank the Welch Foundation, the Searle Scholars Program, the Center for Macromolecular Design (TAMU), the Camille-Dreyfus Teacher Scholar Program, and the National Institutes of Health (R01 GM51105) for financial support.

Supplementary Material Available: Synthetic details, mass spectral data, circular dichroism spectra for **1** and **2**, an analysis of the binding of **1** to **2** accomplished by fitting experimental CD data to the laws of mass action, HPAC analyses of the binding of **2** and related peptides to immobilized **1**, and temperature effects and salt effects upon the **1**−**2** complex in solution (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JA943080D

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