A Chemical Model of a Protein β-Sheet Dimer

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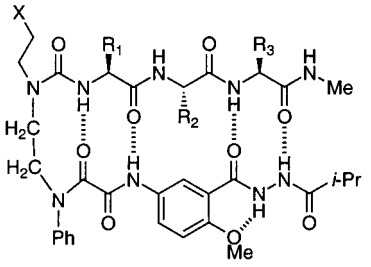
β-Sheet formation is an important form of protein interaction that is involved in protein dimerization, recognition between different proteins, and protein aggregation.[1] Proteins that function as β-sheet dimers (or higher oligomers) include HIV-1 protease, many lectins, and the defensins (Figure 1). An attractive approach to modulating the function of these protein β-sheet dimers involves developing synthetic molecules that can mimic or interrupt β-sheet dimer formation.[2] This paper reports our first efforts directed toward this goal: a chemical model of a protein β-sheet dimer.

We have previously developed monomeric chemical models of protein β-sheets (artificial β-sheets) in which molecular templates induce β-sheet structure in attached peptide strands.[3−5] These templates include an oligourea *molecular scaffold*, designed to hold multiple peptide or peptidomimetic strands in proximity, and a 5-amino-2-methoxybenzoic acid β-strand mimic, designed to duplicate the hydrogen-bonding functionality of one edge of a peptide β-strand. In the present study, we combine a peptide strand, a diurea template, and the β-strand mimic with a new group, an oxalamide linker, to form artificial β-sheets **1**. In



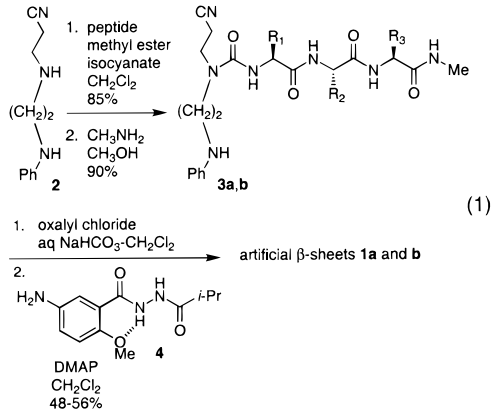
1a $R_1 = R_{Phe}$, $R_2 = R_{Ile}$, $R_3 = R_{Leu}$, X = CN
1b $R_1 = p\text{-}NO_2\text{-}C_6H_4CH_2$, $R_2 = R_{Val}$, $R_3 = R_{Met}$, X = CN
5 $R_1 = R_{Tyr}$, $R_2 = R_{Ile}$, $R_3 = R_{Lys}$, $X = NH_3^+ \ CF_3CO_2^-$

contrast with our previous artificial β-sheets, the β-strand mimic is on the *bottom* edge of the β-sheet. Also in contrast with our previous compounds, artificial β-sheets **1** form dimers with well-defined structures.

Artificial β-sheet **1a** was prepared from diamine **2**[6] as shown in eq 1. Reaction of diamine **2** with phenyalanylisoleucylleucine methyl ester isocyanate[7] and aminolysis of the methyl ester group of the resulting urea adduct with methylamine afforded peptide methylamide urea adduct **3a** in 77% yield. Treatment of this compound with oxalyl chloride and reaction of the resulting

Figure 1. Ribbon diagram of defensin HNP-3.[3] (PDB reference 1dfn).[4]

oxamoyl chloride adduct with amine **4**[8] then afforded artificial β-sheet **1a** in 48−56% yield. Artificial β-sheet **1b**, which contains *p*-nitrophenylalanine, valine, and methionine, in place of phenylalanine, isoleucine, and leucine, was prepared in a similar fashion and with comparable yields.



(1)

[1]H NMR transverse-ROESY (Tr-ROESY)[9] studies show that **1a** adopts an intramolecularly hydrogen-bonded β-sheet structure in $CDCl_3$ solution (10 mM, 30 °C). Notably, **1a** exhibits interstrand ROEs between H_6 of the β-strand mimic and the isoleucine α-proton and side-chain protons. Additional interstrand ROEs occur between the terminal methylamide and isobutryryl groups, the leucine side-chain and isobutyryl group, the oxalamide and urea NH groups, and the leucine and hydrazide NH groups.

Of particular significance is an ROE between the phenylalanine and leucine α-protons. This ROE is not consistent with a monomeric β-sheet structure and suggests the formation of an antiparallel β-sheet dimer. Figure 2 shows the structure of this dimer and illustrates all of these interstrand ROEs. Figure 3 provides a molecular model of this structure that is consistent with these ROEs.

To confirm that the ROEs between the phenylalanine and leucine α-protons of **1a** are *intermolecular* and result from dimer formation, we performed a crossover experiment consisting of Tr-ROESY studies of a mixture of **1a** and **1b**. These studies reveal intersheet ROEs between the phenylalanine α-proton of **1a** and the methionine α-proton of **1b** and between the leucine α-proton

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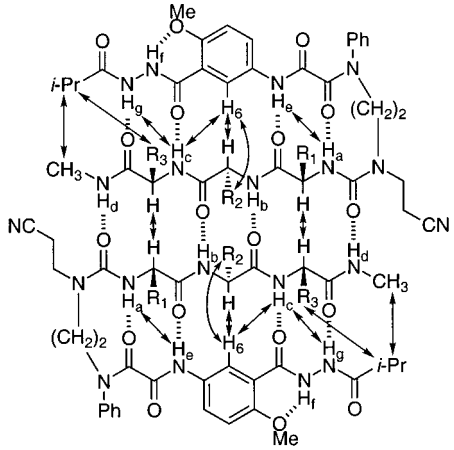

Figure 2. Key interstrand ROES (represented by arrows) in the dimer of artificial β-sheets **1a**.

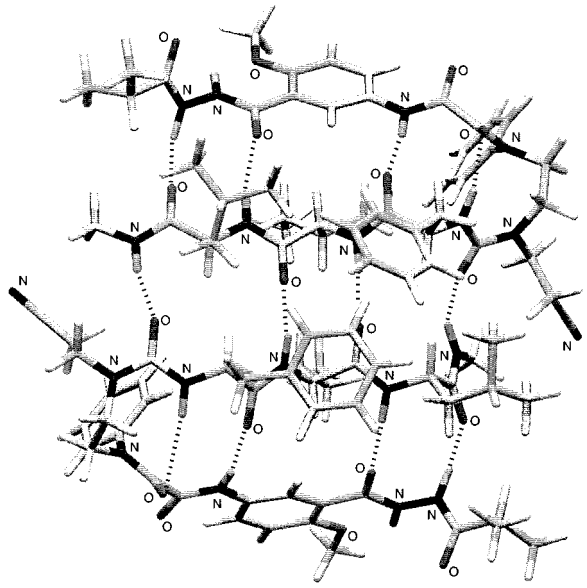

Figure 3. Model of artificial β-sheet **1a**, generated using MacroModel V6.5 with the AMBER* force field. The starting geometry (before minimization) was chosen to reflect ^{1}H NMR ROE and coupling constant data, which suggest a preferred gauche conformation of the 1,2-diaminoethane diurea backbone. The starting conformations of the amino acid side chains are largely arbitrary.

of **1a** and the nitrophenylalanine α-proton of **1b**, which result from the **1a·1b** heterodimer. Intersheet ROEs associated with the **1a·1a** and **1b·1b** homodimers (Phe α-Leu α and nitroPhe α-Met α) are also present.

^{1}H NMR titration studies confirm that **1a** dimerizes and support the model represented by Figures 2 and 3. Thus, the chemical shifts of the isoleucine and methylamide NH protons (H_b and H_d) shift substantially downfield with increasing concentration in $CDCl_3$, while the other NH protons exhibit considerably less shifting (Figure 4). Similarly, the phenylalanine and leucine α-protons shift downfield with increasing concentration in $CDCl_3$, while the isoleucine α-proton does not.[10] Analysis of the NH and α-proton shift data by nonlinear least-squares fitting of a dimerization isotherm reveals a dimerization constant of 600 M^{-1}.[11] The NH and α-protons of **1b** exhibit similar patterns of downfield shifting and a dimerization constant of 90 M^{-1}.

To evaluate the effect of water upon the structure and dimerization properties of the artificial β-sheets, we prepared artificial β-sheet **5**. This water-soluble analogue of **1a** contains

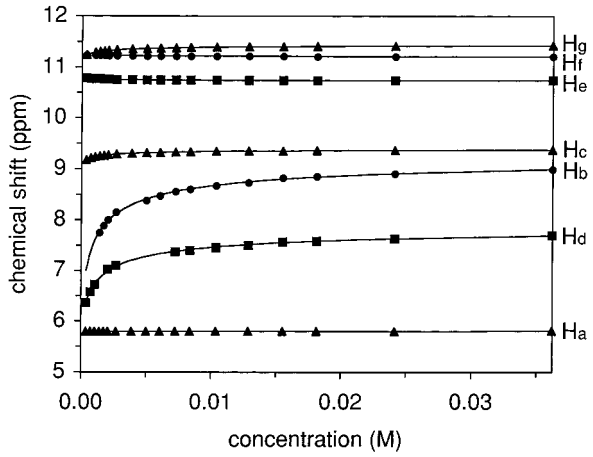

Figure 4. ^{1}H NMR chemical shift of NH protons of **1a** as a function of concentration in $CDCl_3$ solution at 25 °C.

tyrosine instead of phenylalanine (R_1), lysine instead of leucine (R_3), and an ammonium group instead of a cyano group (X). In D_2O, this compound exhibits an interstrand ROE between H_6 of the β-strand mimic and the isoleucine α-proton but does not exhibit an intersheet ROE between the tyrosine and lysine α-protons. It also does not show concentration-dependent downfield shifting of the tyrosine and lysine α-protons. Collectively, these data indicate that **5** can fold into a β-sheet but does not dimerize significantly in dilute aqueous solution.[12]

The development of molecules that self-assemble into well-defined dimers constitutes an important research area that has received considerable attention during the 1990s.[13] Although models of β-sheets[14] that dimerize[15] or interact through β-sheet formation[16] have been reported, the current system is, to our knowledge, the first that forms a well-defined multistranded β-sheet dimer that resembles the defensins and other protein β-sheet dimers. We envision this model system as a platform with which to study the β-sheet dimerization of proteins. In subsequent studies we will address questions of how to achieve dimerization of artificial β-sheets in aqueous solution and how to inhibit β-sheet dimer formation among proteins.

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Supporting Information Available: Synthetic procedures and PFG COSY and Tr-ROESY spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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