Programmed Self-Sorting of Coiled Coils with Leucine and Hexafluoroleucine Cores

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Specific protein−protein interactions are crucial for virtually all biological processes.[1] Naturally occurring surfaces that mediate protein−protein interactions usually contain either elements of polar specificity, for example hydrogen bonding or salt bridges, or complementary hydrophobic patches that contain side chains that maximize van der Waals interactions.[2] We,[3] and others[4] have recently described a new type of protein−protein interaction interface that could potentially be both hydrophobic and lipophobic. This is accomplished by the introduction of nonproteinogenic, fluorine-containing amino acids.[5,6] Reported here is the design, synthesis, and programmed self-sorting of peptide systems with orthogonally miscible hydrocarbon and fluorous (highly fluorinated) cores.

Peptides **H** and **F** are equipped with N-terminal cysteine residues and were designed to form parallel homodimeric coiled coil assemblies.[7] These peptides have an identical sequence except that all seven of the core leucine residues in **H** have been replaced by 5,5,5,5′,5′,5′-α-*S*-hexafluoroleucine in **F** (Figure 1), shielding 28 trifluoromethyl groups from aqueous solvent in the canonical fluorinated dimer. Hexafluoroleucine was synthesized according to a recently documented procedure.[8] The peptides were assembled on 4-methylbenzhydrylamine (MBHA) resin according to the in situ neutralization protocol for *t*-Boc peptide synthesis as described previously[9] and purified by reverse-phase HPLC. Purity of the peptides was confirmed by analytical HPLC and MALDI mass spectrometry. **H** and **F** are designed to form parallel coiled coil structures due to unfavorable interhelical electrostatic interactions in the antiparallel arrangements.[10] Furthermore, a single polar residue Asn14 was incorporated in the hydrophobic core which can only hydrogen bond in the parallel arrangement.[11] The peptides are equipped with a Gly-Gly-Cys tripeptide at the NH$_2$-terminus. The cysteine residue permits redox chemistry in the form of disulfide−thiol equilibrium, and the two glycine residues provide a flexible linker. Disulfide bonded dimers of **H** (**HH**) and **F** (**FF**) were synthesized by air oxidation of the monomeric peptides in pH 8.50 Tris buffer.

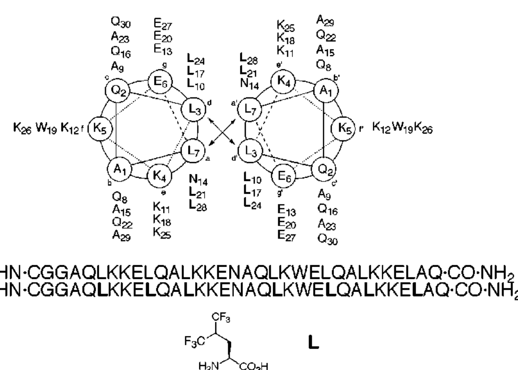

Figure 1. Helical wheel representation of residues 1−30 of **H** and **F** looking down the superhelical axis from the N terminus. All seven core leucines in **H** were replaced by hexafluoroleucine (**L**) in **F**.

The preference for sorting into homodimeric populations under equilibrium conditions was examined by a disulfide-exchange assay.[12] Preformed disulfide bonded heterodimer **HF** was incubated in a pH 7.50 redox buffer at 20 °C, conditions under which disulfide exchange is rapid. Aliquots were removed from the reaction at various times and quenched with 5% trifluoroacetic acid. The time points were then analyzed by analytical reverse-phase HPLC. Relative concentrations of the disulfide-bonded hetero- and homodimers were estimated by integration of the area under corresponding peaks at 230 nm. Within 30 min of the start of the reaction, the heterodimer disproportionates into the two homodimers **HH** and **FF**.[13] After 200 min, only a trace of the heterodimer (∼3%) remains (Figure 2). Further change in the reaction mixture was not observed even after 18 h. Assuming that the glycyl linkers allow the cysteines to exchange randomly under redox buffer conditions, the data indicate that the homodimers are preferred over the heterodimer by 26-fold. To establish that the reaction had reached equilibrium, we placed an equimolar amount of the reduced peptides **H** and **F** under similar redox buffer conditions and monitored the reaction for 18 h. Again, the heterodimer accounted for only 3% of all disulfide-bonded species. Unambiguous stepwise synthesis of the heterodimer **HF** confirms that the disulfide bond-forming chemistry is reversible and under thermodynamic control, and that there are no kinetic barriers to the formation of the disulfide-bonded heterodimer **HF**.[14] It would appear that the peptides **H** and **F** are predisposed to form homodimers.[15] Instability of the heterodimer and the hyperstability of the fluorinated dimer provide the driving force for preferential homodimer formation. From the peak ratios

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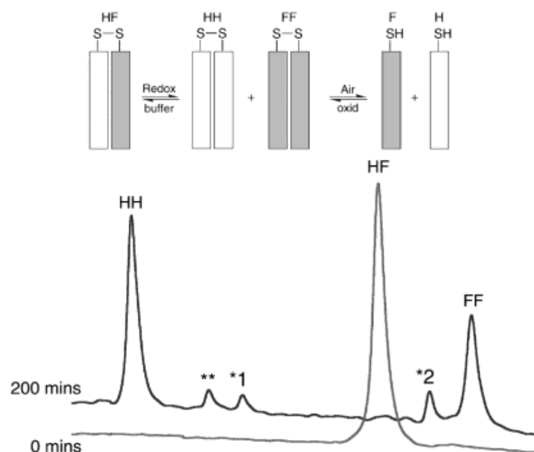

Figure 2. HPLC traces showing preferential homodimer formation by fluorous and hydrocarbon cores. Preformed disulfide-bonded heterodimer **HF** (25 μM) was incubated in redox buffer.[17] After 200 min, only homodimers and mixed disulfides remain. The mixed heterodimer is estimated to be less than 3% of all **H** and **F** containing peptides at equilibrium. Peaks marked *1 and *2 are **H** monomer and **F**-glutathione mixed disulfide respectively and ** is an impurity.[18]

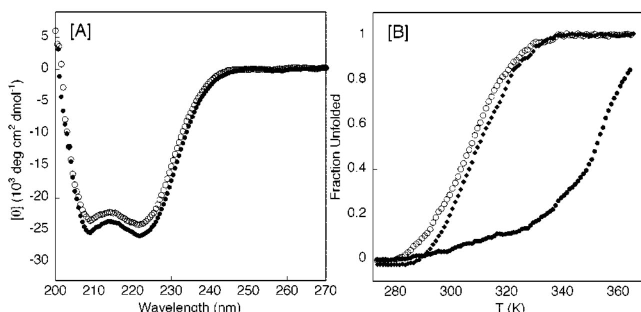

Figure 3. (A) Circular dichroism spectra of **HH** (○) and **FF** (●). Conditions: [**HH**] = [**FF**] = 2 μM, pH 7.40, 137 mM NaCl, 2.7 mM KCl, 10 mM PBS, 10 °C. (B) Thermal denaturation profiles of **HH** (○), **FF** (●) and **HF** (◆). Conditions: [**HH**] = [**FF**] = [**HF**] = 2 μM, 5 M Gdn·HCl, pH 7.40, 137 mM NaCl, 2.7 mM KCl, 10 mM PBS.

at equilibrium, the free energy of specificity for the formation of homodimers, ΔG_{spec} is calculated to be at least −2.1 kcal/mol.[16]

Circular dichroism spectra of peptides **HH**, **HF**, and **FF** reveal the α-helical character of all three disulfide-bonded dimers with characteristic minima at 208 and 222 nm (Figure 3A). The order of stability is readily established when melting curves as monitored by CD are compared. All three peptides **HF**, **HH**, and **FF** display cooperative unfolding transitions as a function of temperature in the presence of guanidine hydrochloride (Gdn·HCl). The melting temperatures in 5 M Gdn·HCl of **HH** (34 °C) and that of **HF** (36 °C) are very similar. In contrast, the fluorinated peptide **FF** melts at an estimated 82 °C under these conditions (Figure 3B). The fluorinated disulfide-bonded dimer is remarkably stable, resisting even minimal denaturation at 6 M Gdn·HCl at room temperature. Even at 7 M Gdn·HCl concentration, **FF** resists thermal denaturation up to 45 °C (Table 1). Thus, the fluorinated assembly **FF** is significantly more stable than either the heterodimer **HF** or the hydrocarbon homodimer **HH**. A priori, the

(16) The free energy of specificity was calculated based on a thermodynamic cycle (see Supporting Information).

Table 1. Melting Temperatures and Solution MW for Disulfide-Bonded Dimers

peptide	T_m (°C)[a]	MW_{app} (no. of helices)[c]
HH	34	7501 ± 38 (2)
HF	36	8815 ± 63[d] (2)
FF	82 (45[b])	17835 ± 75 (4)

[a] Determined by monitoring the molar ellipticity at 222 nm as a function of temperature. Conditions: 2 μM peptide, pH 7.40, 5 M Gdn·HCl, 10 mM PBS. [b] In 7 M Gdn·HCl, pH 7.40, 10 mM PBS. [c] Determined by sedimentation equilibrium. Conditions: 15 μM peptide, pH 7.40, 10 mM PBS, 10 °C. [d] Non-random residuals.

T_m of the heterodimer can be expected to be the average of the T_m values of the homodimers (ΔT_m = 0 °C).[19] Differences in ΔT_m have been invoked to explain the specificity of the heterodimeric Fos−Jun peptide pair.[20] In our case, ΔT_m is −22 °C, indicating that the thermal stability of the heterodimer is appreciably lower than the expected intermediate stability. The thermodynamic consequence of the relative stability of the fluorinated peptide assembly **FF** and the instability of **HF** is to shift the equilibrium away from the heterodimer to the homodimers.

Sedimentation equilibrium analysis of the disulfide-bonded dimers in the 2−15 μM range revealed that **HH** has an apparent molecular weight of 7501 D in solution, consistent with two helices forming the coiled coil structure (Table 1). In contrast, **FF** sediments with an apparent molecular weight of 17835 D. This could be due to much larger association constant of **FF** monomers or due to the larger size of the core formed by hexafluoroleucine, forcing it to adopt a coiled coil structure with four helices.

In summary, we have demonstrated the incorporation of hexafluoroleucine as the sole hydrophobic core residue in a designed coiled coil. Furthermore, this is the first example of a very highly specific protein−protein interaction based on the substitution of the hydrophobic core with fluorinated residues. These experiments offer the exciting possibility of design and manipulation of specific helix−helix interactions within the context of the nonpolar environment of membranes.[21] Studies along these lines are currently being pursued in our laboratories and will be reported shortly.

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Supporting Information Available: Experimental details, MALDI mass spectra of **HH**, **HF**, and **FF** (PDF). This material is available free of charge at http://pubs.acs.org.

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(17) Buffer: 125 μM oxidized glutathione, 500 μM reduced glutathione, pH 7.50, 100 mM NaCl, 200 mM MOPS.

(18) Analysis of the reaction mixture by LC-ESI-MS showed that peak marked **HH** contains some **H**-glutathione mixed disulfide (∼10% of peak). Peak **FF** contains some monomeric **F** (∼2% of peak). There is a difference in the extinction coefficients of **HH** and **FF** under the HPLC conditions ($\epsilon_{FF}/\epsilon_{HH}$ = 0.75).

(19) The specificity for heterodimer formation can be approximated by $\Delta T_m = T_m$(heterodimer **HF**) − 1/2 [T_m(homodimer **HH**) + T_m(homodimer **FF**)] = −22 °C.[20]

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