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Reversible α-helix formation controlled by a hydrogen bond surrogate

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ABSTRACT

Strategically placed covalent linkages have been shown to stabilize helical conformations in short peptide sequences. Here we report the synthesis of a stabilized α-helix that utilizes an internal disulfide linkage. Structural analysis indicates that the dynamic nature of the disulfide bridge allows for the reversible formation of an α-helix through oxidation and reduction reactions.

1. Introduction

Methods that provide reversible control over protein secondary structure formation have proven to be useful for probing protein structure and interactions.[1–4] In this report, we describe the design of a helix stabilized by a reversible covalent linkage. The strategy builds on our reported efforts to prepare internally-constrained α-helix mimetics, termed hydrogen bond surrogate (HBS) helices.[5,6] HBS helices contain a covalent bond in place of an intrastrand hydrogen bond between main chain atoms in canonical α-helices (Fig. 1a). We have previously demonstrated that alkene, alkane, and thioether linkages can serve to nucleate and stabilize the desired conformation in short peptides.[7–9] Herein we show that replacement of the main chain hydrogen bond with a disulfide group provides facile access to reversibly stabilized α-helices (Fig. 1a).[1,10,11] The disulfide linkage enforces slightly different dihedral requirements than the hydrocarbon bridges;[12] however, our biophysical studies suggest that conformational stability of dsHBS helix is similar to those of HBS helices. dsHBS helices are readily formed by oxidizing bis-thiol peptides in which the two sulfhydryl groups formally occupy the *i* and *i*+4 positions (Fig. 1b).

2. Results and discussion

For these preliminary studies we designed a short helix based on the p53 activation domain.[13,14] The bis-thiol p53 peptide **1** was synthesized on solid phase as shown in Scheme 1. Peptide **3** was

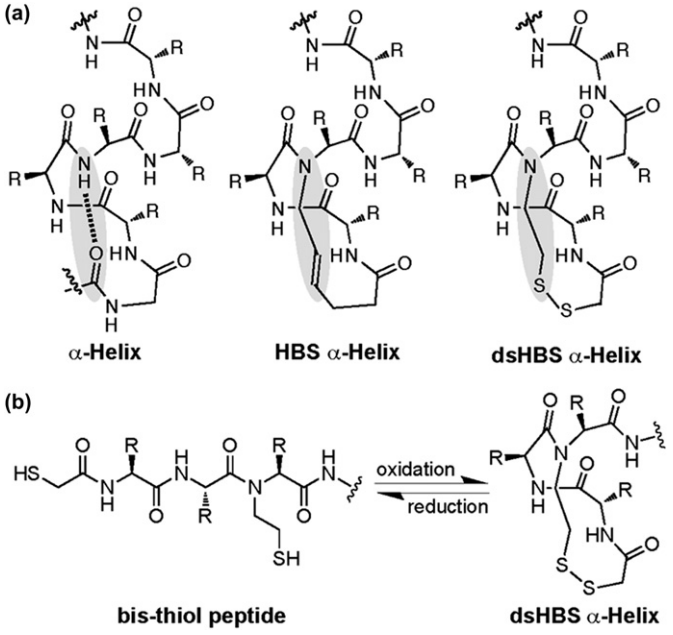

Fig. 1. (a) Hydrogen bond surrogate (HBS) α-helices feature a covalent bond in place of the intramolecular hydrogen bond between the *i* and *i*+4 residues. (b) In dsHBS α-helices the main chain hydrogen bond is replaced with a disulfide linkage, and can be reversibly formed from a bis-thiol peptide.

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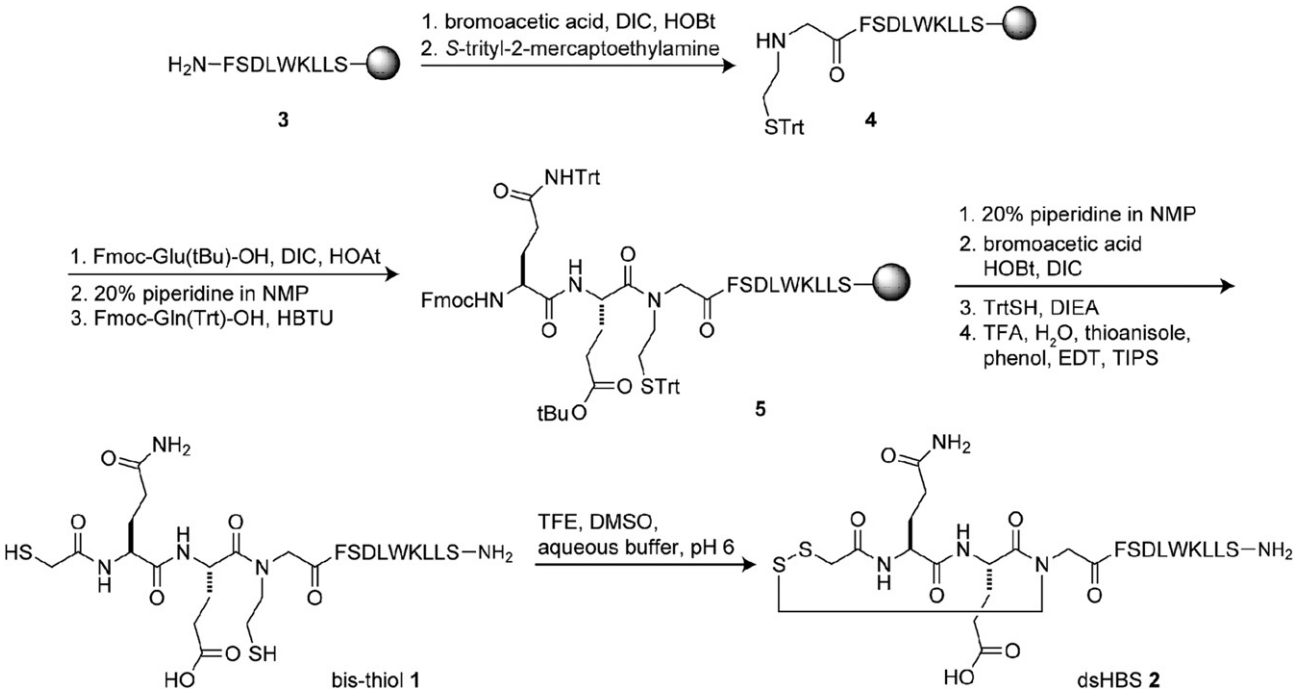

Scheme 1. Solid phase synthesis of bis-thiol **1** and dsHBS α-helix **2**.

prepared using Fmoc chemistry on Rink amide resin.[15] Coupling of **3** with bromoacetic acid followed by treatment with *S*-trityl-2-mercaptoethylamine provided **4**. Sequential coupling of Fmoc-Glu(O^{t}Bu)–OH, Fmoc-Gln(Trt)–OH, and bromoacetic acid, treatment with triphenylmethyl mercaptan followed by cleavage from resin afforded bis-thiol peptide **1**. We evaluated the potential of iodine[10] and DMSO[16] to oxidize HPLC-purified **1** to dsHBS **2** and found DMSO in 10% TFE/ammonium bicarbonate buffer (pH 6) to provide efficient conversion to the desired product (Fig. 2).

The conformation of the peptides was examined by circular dichroism and 2D NMR spectroscopies. CD studies were performed in dilute phosphate buffered saline to obtain a measure of their helical content (Fig. 3). CD spectroscopy suggests that, as expected, bis-thiol **1** is weakly helical in aqueous solutions while dsHBS **2** provides a CD signature typical of a canonical α-helix, with a double minima near 208 and 222 nm and a maximum at 190 nm.[17–21] Upon treatment with 1.5 equiv of tris(2-carboxyethyl)phosphine (TCEP), a reducing agent, dsHBS **2** loses its conformational preference. The CD traces of the bis-thiol peptides are similar to that of linear p53 peptide analog (**6**: Ac-QEGFSDLWKLLS-NH_2).

We performed 2D NMR experiments to further establish the conformation of **2**. NMR studies were performed in 20% TFE-d_3 in PBS (pH 3.5) on a Bruker 500 MHz spectrometer. The organic co-solvent was needed to solubilize the compound at the low millimolar concentrations needed for NMR studies. Trifluoroethanol, along with other organic co-solvents, is known to enhance helicity of peptides.[22–24] The %helicity of **2** increases by 1.5-fold in the presence of 20% TFE as compared to the pure aqueous solution according to CD spectroscopy (Supplementary data). The shapes of the curves, as well as the 208/222 nm ratio used to evaluate α-helicity remain constant in the two solvents.[17,18] Thus, CD spectroscopy suggests that a different helical conformation is not being stabilized by the addition of the co-solvent.

A set of 2D TOCSY and NOESY spectra was used to assign ^{1}H NMR resonances for **2**. Sequential NH–NH (i and i +1) NOESY cross-peaks, a signature of helical structure, were observed for **2** as depicted in Fig. 4. The NOESY spectrum further reveals medium to weak (i, i+3) and (i, i+4) CHα–NH cross peaks that support an α-helical conformation in **2**, although spectral overlap prevented assignment of some key cross-peaks. The NMR spectra, peak assignments, and the NOESY correlation chart are included in the Supplementary data.

3. Conclusion

We have developed a new class of hydrogen bond surrogate helices in which the conformation can be reversibly controlled through oxidation of bis-thiols or reduction of the resulting disulfide bridge. We find that DMSO-mediated oxidation provides efficient conversion to the desired compound. CD and NMR spectroscopies provide strong support for our hypothesis that the disulfide bridge provides conformationally-defined α-helices. In ongoing efforts we are utilizing dsHBS helices to probe biophysical parameters related to the helix-coil transition; the results of these studies will be reported in due course.

4. Experimental section

4.1. General

Commercial-grade reagents and solvents were used without further purification, except as indicated. Dry DMF was obtained using an Innovative Technology PureSolv solvent drying system. All reactions were either stirred or mechanically shaken at room temperature, except as indicated. After each step, the resin was sequentially washed with DMF (3×5 mL), MeOH (3×5 mL), and DCM (3×5 mL). Microwave irradiation was performed in the CEM Discover single-mode reactor with controlled power, temperature, time, and stirring settings. NMR experiments were performed using a Bruker AVANCE 500 MHz spectrometer. Reversed-phase HPLC experiments were conducted with 4.6×150 mm (analytical scale) or 21.4×150 mm (preparative scale) Waters C_{18} Sunfire columns