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Helix Propensity of Highly Fluorinated Amino Acids

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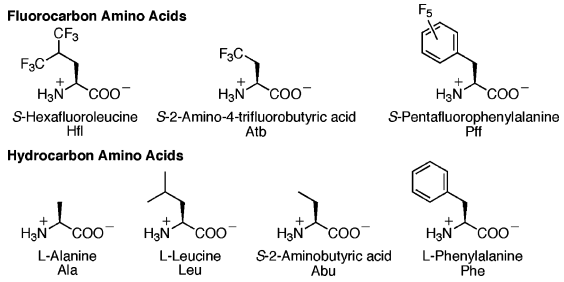
Highly fluorinated amino acids have been used to stabilize proteins[1−7] for potential application in various protein-based biotechnologies, such as protein therapeutics, industrial scale biotransformations, and biosensors. To enhance protein stability, natural hydrocarbon amino acids have been substituted with highly fluorinated amino acids.[1−7] The effect of enhanced protein stability upon replacing hydrocarbon residues with fluorocarbon residues is referred to as the fluoro-stabilization effect.[1−7] In particular, replacing leucine (Leu) with (*S*)-5,5,5,5′,5′,5′-hexafluoroleucine (Hfl, Chart 1) has enhanced the stability of several helical proteins by 0.32−0.83 kcal·mol^{-1}·residue^{-1} for each substitution.[3,5−7] The enhanced stability has been attributed to the higher hydrophobicity of the fluorocarbon side chain compared to that of the natural hydrocarbon side chain,[6] assuming that the helix propensities are the same for Leu and Hfl. However, substituting Leu with the isosteric (*S*)-2-amino-4,4,4-trifluorobutyric acid (Atb) resulted in destabilization of a helical protein.[8] These pioneering studies have focused on the effect of fluorinated amino acids on the overall stability of the helical proteins. To gain further insight into the effect of these highly fluorinated amino acids on helix formation exclusively, we report the helix propensity of three highly fluorinated amino acids: Hfl, Atb, and (*S*)-pentafluorophenylalanine (Pff).

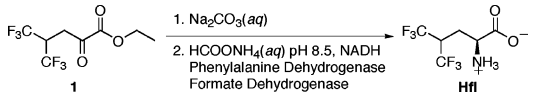
Several syntheses for Hfl have been reported, including one stereoselective,[9] one racemic,[3] and two stereospecific syntheses.[10,11] Instead of following these known procedures, we developed a short chemoenzymatic synthesis of Hfl (Scheme 1). Compound **1** was hydrolyzed to the corresponding acid and subjected to reductive amination mediated by phenylalanine dehydrogenase[12,13] using NADH as the reductant to give the desired Hfl[9] in >99% enantiomeric excess.[14] This stereoselective synthesis of Hfl has an extremely high enantioselectivity due to the high fidelity of the enzyme. To incorporate Hfl into peptides, the Hfl backbone amine was subsequently protected with the 9-fluorenylmethyloxycarbonyl (Fmoc) group.

Armed with this new facile synthesis of Hfl, we synthesized seven alanine-based peptides to measure the helix propensity of the amino acids in monomeric helical peptides with minimal intra-helical side chain−side chain interactions.[15] The general sequence for the peptides was Ac-Tyr-Gly-Gly-Lys-Ala-Ala-Ala-Ala-Lys-Ala-Xaa-Ala-Ala-Lys-Ala-Ala-Ala-Ala-Lys-NH_2 (Xaa = Hfl, Atb, Pff, Ala, Leu, Abu, or Phe), with the amino acid of interest incorporated at the central guest position Xaa for each peptide. Peptides were synthesized by solid phase peptide synthesis using Fmoc-based chemistry with no complications despite reported difficulty for Hfl-containing peptides.[6] After cleavage, peptides were purified by reverse phase high performance liquid chromatography to greater than 98% purity.[14] The association state in solution for

Chart 1. Fluorocarbon and Hydrocarbon Amino Acids



Scheme 1. Chemoenzymatic Synthesis of (*S*)-5,5,5,5′,5′,5′-Hexafluoroleucine



all the peptides was monomeric based on size exclusion chromatography and sedimentation equilibrium experiments.[14] Thus, intermolecular interactions should not contribute to the helical content of the peptides in solution.

The helical content of the peptides was evaluated by circular dichroism spectroscopy (CD) at pH 7 (Figure 1). The CD signal at 222 nm was used to calculate the fraction helix,[15] f_{helix} (Table 1). The f_{helix} of peptides with natural hydrocarbon amino acids at the guest position were similar to published values.[15] Importantly, the f_{helix} was consistently higher for peptides with hydrocarbon amino acids at the guest position than peptides with the corresponding fluorocarbon amino acids (KLeu > KHfl; KAbu > KAtb; KPhe > KPff).

The helix propensity (w) of the amino acids at the guest position was calculated from the f_{helix} of the corresponding peptide based on modified Lifson−Roig theory[16,17] (Table 2). The trend for the helix propensities of the natural hydrocarbon amino acids is similar to previously published results.[15] Importantly, the helix propensity is consistently lower for the highly fluorinated amino acids than the corresponding hydrocarbon amino acids ($w_{Pff} < w_{Phe}$; $w_{Atb} \ll w_{Abu}$; $w_{Hfl} \ll w_{Leu}$). The helix propensity of an amino acid can decrease up to 24-fold upon fluorination ($w_{Atb} \ll w_{Abu}$). One possible reason for the drastic decrease in helix propensity upon introducing fluorine atoms is that the fluorocarbon side chain may be partially or fully buried in the unfolded state and more exposed in the monomeric helix state. This would lead to unfavorable helix formation energetics due to the hydrophobic nature of the fluorocarbon side chain.

Substituting Leu with Hfl decreases the helix propensity by 8-fold (1.15 kcal·mol^{-1}·residue^{-1}) but enhances the overall stability of helical proteins by 0.32−0.83 kcal·mol^{-1}·residue^{-1},[3,5−7] suggesting that the fluoro-stabilization effect more than overcomes the less favorable helix propensity of Hfl. In contrast, substituting Leu with

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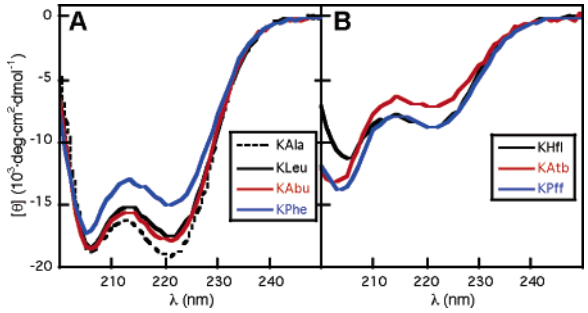

Figure 1. Circular dichroism spectra of the peptides at pH 7 (273 K) in 1 mM phosphate, borate, and citrate buffer with 1 M NaCl. Panel A: KAla, KLeu, KAbu, KPhe. Panel B: KHfl, KAtb, KPff.

Table 1. Mean Residue Ellipticity and Fraction Helix (f_{helix}) of Alanine-Based Peptides

peptide[a]	$[\theta]_{222}$	f_{helix}
KAla	−19100 ± 200	0.594 ± 0.006
KLeu	−17400 ± 200	0.502 ± 0.006
KAbu	−18100 ± 200	0.522 ± 0.006
KPhe	−14800 ± 300	0.426 ± 0.008
KHfl	−8720 ± 200	0.251 ± 0.006
KAtb	−7230 ± 190	0.208 ± 0.005
KPff	−9170 ± 190	0.264 ± 0.006

[a] Peptide KXaa: Ac-Tyr Gly Gly Lys Ala Ala Ala Ala Lys Ala Xaa Ala Ala Lys Ala Ala Ala Ala Lys-NH_2. Guest position Xaa: Ala, Leu, Abu, Phe, Hfl, Atb, or Pff.

Table 2. Helix Propensity (w) and Free Energy of Helix Formation (ΔG) for Various Amino Acids with Hydrocarbon or Fluorocarbon Side Chains

amino acid	w	ΔG (kcal·mol^{-1})[a]
Ala	1.46 ± 0.01	−0.206 ± 0.004
Leu	1.06 ± 0.12	−0.0317 ± 0.0654
Abu	1.22 ± 0.14	−0.108 ± 0.066
Phe	0.636 ± 0.081	0.246 ± 0.074
Hfl	0.128 ± 0.023	1.12 ± 0.11
Atb	0.0513 ± 0.0151	1.61 ± 0.19
Pff	0.154 ± 0.025	1.02 ± 0.10

[a] $\Delta G = -RT\cdot\ln(w)$.

Atb not only decreases the helix propensity by 21-fold (1.64 kcal·mol^{-1}·residue^{-1}) but also decreases the overall stability of the protein,[8] suggesting that the penalty to form a helix is too large to overcome by the fluoro-stabilization effect.

The fluoro-stabilization effect has been attributed to hydrophobics because the difference in hydrophobicity between Leu and Hfl (0.4 kcal·mol^{-1}·residue^{-1})[6] is similar to the change in overall helical protein stability upon substituting Leu with Hfl (0.32−0.83 kcal·mol^{-1}·residue^{-1}).[3,5−7] This estimation of the fluoro-stabilization effect assumes that the helix propensities are the same for the two amino acids. However, our results show that Hfl is significantly less favorable than Leu for helix formation. Therefore, the fluoro-stabilization effect is larger than the previous estimation of 0.32−0.83 kcal·mol^{-1}·residue^{-1} by 1.15 kcal·mol^{-1}·residue^{-1} for Hfl. This would imply that the fluoro-stabilization effect may be more than just hydrophobics but may also include more specific interactions such as those involving the dipole moments of the trifluoromethyl groups.[6,7,18]

In this communication, we have demonstrated a short chemoenzymatic synthesis of Hfl with extremely high enantioselectivity. Furthermore, we have measured the helix propensity of several highly fluorinated amino acids, showing that the helix propensity of natural hydrocarbon amino acids decreases significantly upon fluorination. This difference in helix propensity has previously been overlooked in estimating the magnitude of the fluoro-stabilization effect, resulting in a gross underestimation. To realize the full potential of the fluoro-stabilization effect, the fluorocarbon amino acids, such as Hfl, may need to be incorporated into non-helical structures (i.e., β-sheet). Indeed, the stability of a protein has been enhanced up to 1.44 kcal·mol^{-1}·residue^{-1} by substituting valine with trifluorovaline at a buried position in a β-sheet,[19] highlighting the context dependence of the fluoro-stabilization effect. Alternatively, fluorocarbon amino acids with high helix propensity will need to be developed. The full potential of the fluoro-stabilization effect will provide even more stable proteins for potential applications in various protein-based biotechnologies.

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Supporting Information Available: Experimental details for the synthesis and characterization of hexafluoroleucine and the seven peptides and experimental methods for circular dichroism spectroscopy and calculating the helix propensity for the amino acids. This material is available free of charge via the Internet at http://pubs.acs.org.

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