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Short Communication

Effects of single substitutions with hexafluoroleucine and trifluorovaline on the hydrophobic core formation of a heterodimeric coiled coil

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ABSTRACT

Structural modifications of peptides and proteins using fluorinated amino acids provide the opportunity to modulate their biophysical and pharmaceutical properties. Systematic investigations based on model systems that mimic natural protein–protein interaction domains, such as the coiled-coil folding motif, can provide valuable insights into the behaviour of side chain fluorinated amino acids in natural protein environments. Here, we report the incorporation of hexafluoroleucine and two trifluorovaline stereoisomers at two different hydrophobic core positions of an established parallel heterodimeric coiled-coil model system to evaluate the impact of these substitutions on coiled-coil structure and stability. All of the resulting fluorinated peptides form stable α-helical bundles, and the single substitution of leucine with hexafluoroleucine leads to an increase in thermal stability.

1. Introduction

The incorporation of non-natural amino acids containing heteroatoms that are absent from the pool of canonical amino acids has been a powerful tool in modulating various properties of peptides, such as membrane permeability, protease stability and the specificity and biological activity of peptide-based drugs [1,2]. In this context, fluorine substitution has become an efficient strategy [2–4], the effects of which are due to fluorine's unique properties including very low polarizability and the strongest inductive effect of all the elements [5]. Fluorine has the second smallest atomic radius, thus, the substitution of a hydrogen atom with a fluorine atom can be considered as "shape-conservative" or bioisosteric [6–8]. However, the presence of even a single fluorine atom can significantly alter the hydrophobicity and acidity/basicity of amino acids, which can in turn have dramatic effects on the molecular recognition and conformation of peptides and proteins [3].

Due to its well-studied structure and diverse biological functions, the coiled-coil folding motif has been established as a model system for evaluating the impact of fluorination on hydrophobic protein–protein interactions [9]. The primary structure of an α-helical coiled coil is based on the heptad repeat, a (pseudo-) repetitive pattern of seven amino acids, denoted $(abcdefg)_n$, where the a- and d-positions that are located along one side of the helix are predominantly occupied by hydrophobic residues. When two such helices associate, these residues enable hydrophobic core formation and oligomerization. The e- and g-positions are commonly charged and are involved in interhelical electrostatic interactions that further contribute to the stability and folding specificity of the coiled coil. Positions b, c, and f are solvent exposed and therefore usually contain hydrophilic residues [10].

Several studies have demonstrated that coiled coils in which the hydrophobic core is globally substituted with fluorinated analogues of hydrophobic amino acids, namely leucine and valine, exhibit increased thermal stabilities [3,7,8,11–18]. In contrast, work from our group, using minimally fluorinated building blocks, has revealed that a single amino acid substitution within the hydrophobic core can lead to thermal destabilization [9,19,20], and that the degree of destabilization depends on how efficiently the fluorinated residue packs against neighbouring side chains [21–24]. In previous studies hexafluoroleucine [6–8,13,15–17] and trifluorovaline [18] were used for extensive modifications of

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coiled-coil peptides. In order to address the question of whether single substitutions with highly fluorinated side chains can restore or enhance the thermal stability of such structures, we now report the substitution of leucine with 5,5,5,5′,5′,5′-hexafluoroleucine (5^3,$5'^3$-F_6Leu), and the substitution of valine with two different diastereomers of 4,4,4-trifluorovaline (4^3-F_3Val) in a parallel heterodimeric coiled-coil model system.

2. Results and discussion

To evaluate the impact of single substitutions of leucine and valine with their fluorinated analogues on coiled-coil interactions, we singly incorporated either 5^3,$5'^3$-F_6Leu or the two 4^3-F_3Val stereoisomers into positions d_{19} and a_{16}, respectively, of the previously established VPE/VPK model system (Fig. 1), and investigated the structure and stability of the resulting heterodimers by means of CD spectroscopy and size exclusion chromatography in combination with static light scattering (SEC–SLS).

We find that the dimeric oligomerization state of the parent system is retained when any one of the three fluorinated VPK variants assembles with VPE (Table 1 and Supplementary Data). Moreover, the CD experiments reveal that all fluoromodified VPK-analogues form α-helical bundles with VPE, as demonstrated by the two distinct characteristic minima at 208 and 222 nm (Fig. 2); these traces also suggest that the substitution of Val_{16} and Leu_{19} by their fluorinated analogues causes no significant structural

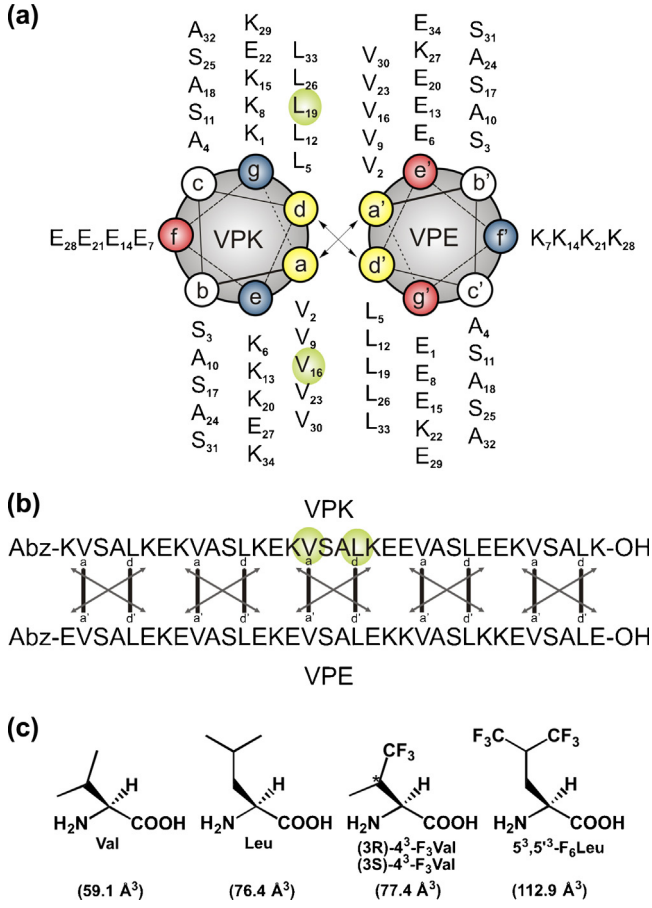

Fig. 1. (a) Helical wheel representation of the parallel VPE/VPK heterodimer [20]. (b) Amino acid sequence of the VPE/VPK system. Substitution positions are highlighted in green. (c) Structures of valine, leucine, and their fluorinated analogues 4,4,4-trifluorovaline (4^3-F_3Val) and 5,5,5,5′,5′,5′-hexafluoroleucine (5^3,$5'^3$-F_6Leu). The vdW volumes given in parentheses correspond to the side chains (starting at C_β), and were calculated according to Zhao et al. [25].

Table 1
Theoretically and experimentally determined molecular weights of VPE/VPK heteromers. Standard deviations were determined from three independent measurements.

Peptide	Theoretical dimer mass (Da)	SEC/SLS determined mass (Da)
VPE/VPK	7576	7578 ± 16
VPE/VPK-5^3,$5'^3$-F_6Leu_{19}	7684	7685 ± 40
VPE/VPK-(3R)-4^3-F_3Val_{16}	7630	7674 ± 121
VPE/VPK-(3S)-4^3-F_3Val_{16}	7630	7634 ± 15

perturbation. In fact, the fluorinated variants appear to have slightly higher helical content compared to the parent VPE/VPK bundle. This observation is somewhat surprising because the known α-helix propensities of the fluorinated amino acids are considerably lower than those of their hydrocarbon parent compounds ($\omega_{Leu} = 0.994 \pm 0.093$ [26]; $\omega_{5^3,5'^3\text{-}F_6Leu} = 0.123 \pm 0.023$ [27]; $\omega_{Val} = 0.411 \pm 0.041$ [26]; $\omega_{4^3\text{-}F_3Val} = 0$ [26]). One possible explanation for this apparent inconsistency is that α-helix propensities of the fluorinated amino acids were measured within the context of a monomeric helical model peptide: thus, the lower ω values for the fluorintaed amino acids may be attributed to less favourable interactions between the relatively more hydrophobic fluorinated residues and the aqueous environment within this particular experimental regime. Obviously, α-helix propensity does not play a dominant role when substitutions are made within the context of a complex quarternary assembly [26].

5^3,$5'^3$-F_6Leu and 4^3-F_3Val are more hydrophobic than their hydrocarbon analogues [26,28], and since coiled-coil formation is driven by the hydrophobic effect, it would be expected that the substitution of a hydrophobic core position with these amino acids lead to a stabilization of the VPE/VPK system [22]. Indeed, on the basis of the melting temperatures, a higher thermal stability is observed for VPE/VPK-5^3,$5'^3$-F_6Leu_{19} (74.4 °C) compared to the parent helix bundle (70.7 °C, Fig. 3); we calculated $\Delta G^{\emptyset}$ values in each case and found that the VPE/VPK-5^3,$5'^3$-F_6Leu_{19} with 11.9 kcal mol^{-1} is indeed somewhat more stable than the parent (11.1 kcal mol^{-1}; Supplementary Data). However, the melting point of VPE/VPK-(3R)-4^3-F_3Val_{16} is similar to that of the parent bundle (70.8 °C vs 70.7 °C), whereas the VPE/VPK-(3S)-4^3-F_3Val_{16}

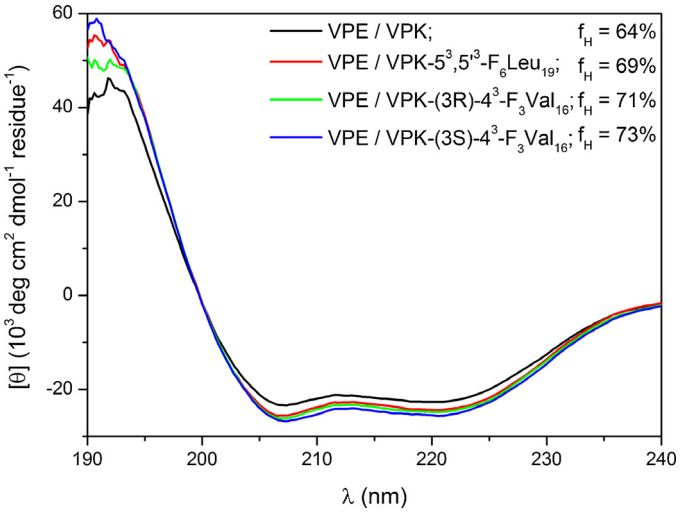

Fig. 2. CD spectra of VPK/VPE heteromers. Helical content for each heteromer (f_H) given in legend. Spectra were recorded at 20 °C in 100 mM phosphate buffer at pH 7.4. The spectra were normalized and represent the mean of three independent measurements at an overall peptide concentration of 20 μM (10 μM in each monomer).