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Selection of a buried salt bridge by phage display

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ABSTRACT

The α-helical coiled coil is a valuable folding motif for protein design and engineering. By means of phage display technology, we selected a capable binding partner for one strand of a coiled coil bearing a charged amino acid in a central hydrophobic core position. This procedure resulted in a novel coiled coil pair featuring an opposed Glu-Lys pair arranged staggered within the hydrophobic core of a coiled coil structure. Structural investigation of the selected coiled coil dimer by CD spectroscopy and MD simulations suggest that a buried salt bridge within the hydrophobic core enables the specific dimerization of two peptides.

The α-helical coiled coil is one of the most widespread structural motifs in nature and is found in motor proteins, transcription factors, viral fusion proteins, and many more.[1] Due to extensive investigations within the last decades, the coiled coil is also one of the best understood protein folding motifs.[2] The structural simplicity and distinctive coherence between sequence and structure allows the de novo design of coiled coils with special features and makes it a useful tool in protein engineering.[3,4] In such cases, one of its most valuable features is the formation of highly specific dimerization domains, which introduces the possibility of targeting viral fusion proteins or transcription factors in therapeutic applications. Furthermore, the coiled coil has proven to be an excellent model system for the systematic investigation of protein folding.[5,6]

Coiled coils typically consist of two to 5 right-handed α-helices that wrap around each other to form a left-handed superhelix. The primary structure of each helix comprises the so-called heptad repeat, a periodicity of seven residues commonly denoted (a–b–c–d–e–f–g)$_n$. Typically, nonpolar residues occupy positions **a** and **d**, forming a hydrophobic surface which initiates oligomerization under aqueous conditions. Charged amino acids in positions **e** and **g** form a second interaction domain which favors coiled coil formation by interhelical ionic interactions, while positions **c**, **b** and **f** are solvent-exposed and thus often populated by polar residues.

Despite the important role of the hydrophobic character of positions **a** and **d** for the formation and stability of the coiled coil folding motif, an analysis of protein databases revealed that in natural occurring proteins up to 20% of these positions are populated by polar and charged residues.[7] In spite of their destabilizing effect, these amino acids play a decisive role in the oligomerization state and orientation of the monomers within the coiled coil.[8,9] As the construction of specific interacting peptide domains is one of the main goals of coiled coil design, modification of the hydrophobic core by charged amino acids represents a promising strategy for the design of coiled coil heteromers.[10] Buried salt bridges have already been used successfully in the design of highly specific interacting coiled coil dimers.[11–13] However, these approaches are commonly based on lysine or arginine analogues with shortened side chains in order to minimize steric mismatches within the hydrophobic core. Even so, folding motifs which are applicable for synthesis in vivo, desirable for many applications, typically require a composition of naturally occurring amino acids. Otherwise, charged interactions within the hydrophobic core provide a promising design principle to direct oligomerization specificity in a manner which keeps the **e**- and **g**-positions free for the introduction of further specifications. To combine both features (control of heteromerization specificity by charged interactions within the hydrophobic core and accessibility by in vivo synthesis) within one system, we were interested in the determination of peptides built up of canonical amino acids which specifically interact with coiled coil strands bearing charged amino acids in hydrophobic core positions.

In contrast to the often used strategy of rational peptide design, we used saturation mutagenesis to construct an extensive library of potential coiled coil pairs which was screened using phage display technology.[14] This technique links the phenotype of a peptide which is displayed on the surface of a bacteriophage with the

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genotype encoding this peptide within the phage particle. While saturation mutagenesis enables generation of a phage displayed peptide library, physical linkage between phenotype and genotype enables the easy identification of individual peptides which are selected by binding preference to a given target. Phage display has already proven to be a powerful tool for the screening of protein–DNA, protein–protein, and protein–peptide interactions.[15–18] Previously, this technique was successfully applied in the determination of specific coiled coil pairing, demonstrating its suitability for peptide design.[19,20]

The starting point for our studies was the coiled coil pair VPE/VPK, reported by our group to form parallel heterodimers.[21] VPK features lysine in positions **e** and **g** while **e′** and **g′** in VPE are occupied by negatively charged glutamic acid to further the formation of heterodimers by ionic interactions under physiological conditions (Fig. 1a). A parallel arrangement as well as a preference for dimer formation is dictated by valine in position **a**.[22] In order to place a negatively charged side chain within the hydrophobic core, Leu_{19} in VPK was replaced by glutamic acid (Fig. 1b). As expected, this substitution results in significant destabilization of the VPK/VPE coiled coil.

Neither homodimerization of VPK-E_{19} nor the formation of VPK-E_{19}/VPE dimers could be observed by CD spectroscopy (Fig. 2). Subsequently, the four amino acid positions within VPE which directly interact with Glu_{19} in VPK-E_{19} were fully randomized and the resulting VPE-library was fused to the minor coat protein pIII on the surface of the filamentous bacteriophage M13 (Fig. 1b). The DNA fragment that encodes for the VPE-peptide, including the four randomized positions $\mathbf{a_{16}}$, $\mathbf{d_{19}}$, $\mathbf{e_{20}}$ and $\mathbf{a_{23}}$, was inserted into the phagemid vector pComb3H to the 5′-end at the gene that encodes for the C-terminal part of the truncated minor coat protein pIII.[23] After successful cloning of the randomized DNA into M13, amplified phage that present the peptide library were used in the selection for binding partners of the Glu_{19}-substituted VPK-variant. VPK-E_{19} used for the selection procedure carries an N-terminal biotin label for immobilization on streptavidin-coated magnetic beads. Coiled coil pairing selectivity was then used to determine the best binding partner in the library, which is able to compensate for the destabilizing effect of the charge within the hydrophobic domain of VPK-E_{19}.

In a control experiment, five rounds of panning against wild type VPK resulted in a variety of sequences which can be summarized as the motifs VPK-$Z_{16}L_{19}X_{20}L_{23}$ and $L_{16}L_{19}X_{20}Z_{23}$, where X indicates predominantly hydrophobic residues and Z marks aromatic residues (Table S3). Despite the surprising variety of selected VPE-variants, the hydrophobic character of the amino acids which were found in the positions of the hydrophobic core clearly correlate with the design principles of the α-helical coiled coil.

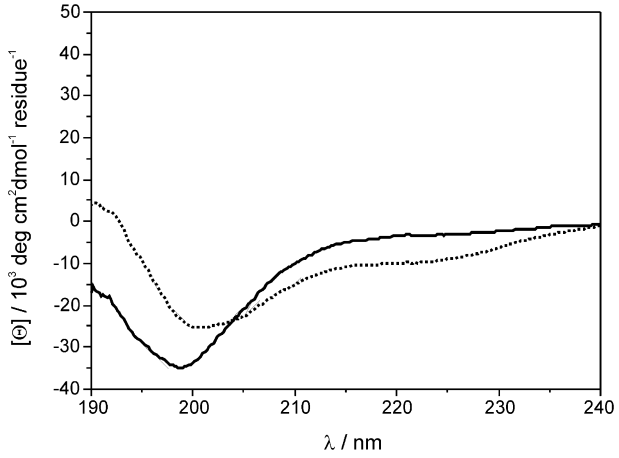

Figure 2. CD spectra of VPK-E_{19} (solid line) and an equimolar mixture of VPK-E_{19} and wild type VPE (dotted line). Spectra were recorded at 20 °C in 100 mM phosphate buffer pH 7.4 at an overall peptide concentration of 20 μM.

In contrast, panning against VPK-E_{19} resulted in greater sequence convergence. Sequencing of nine clones yielded only two different phenotypes: 8 of 9 clones matched the sequence of VPE-LLLK, in which leucine occupies positions $\mathbf{a_{16}}$, $\mathbf{d_{19}}$ and $\mathbf{e_{20}}$ and lysine position $\mathbf{a_{23}}$. The remaining clone differs only in position $\mathbf{e_{20}}$, where methionine was found instead of leucine. Overall, leucine represents the most common amino acid within the hydrophobic core of naturally occurring coiled coil peptides and its selection in positions $\mathbf{a_{16}}$ and $\mathbf{d_{19}}$ is unsurprising. The increase in hydrophobic surface area by the substitution of Glu_{20} with leucine or methionine obviously stabilizes the coiled coil structure even further.

As expected, CD analysis of VPK-E_{19} and VPE-LLLK in isolation yields CD spectra characteristic for predominantly random coil structures (Fig. 3a). In both peptides, the charged residue within the hydrophobic core, in addition to the repulsive interactions between the **e**/**g′** and **g**/**e′** positions which are a feature of the original design strongly discourages the formation of homodimers. In addition, a 1:1 mixture of VPK-E_{19} and wild type VPE does not result in coiled coil formation (Fig. 2). In contrast, an equimolar mixture of VPK-E_{19} and VPE-LLLK shows a strong α-helical CD signal. The melting temperature of the new heterodimer is 36.4 °C compared to 71.3 °C for the VPK/VPE wild type (Ref. 21). Plotting the mean

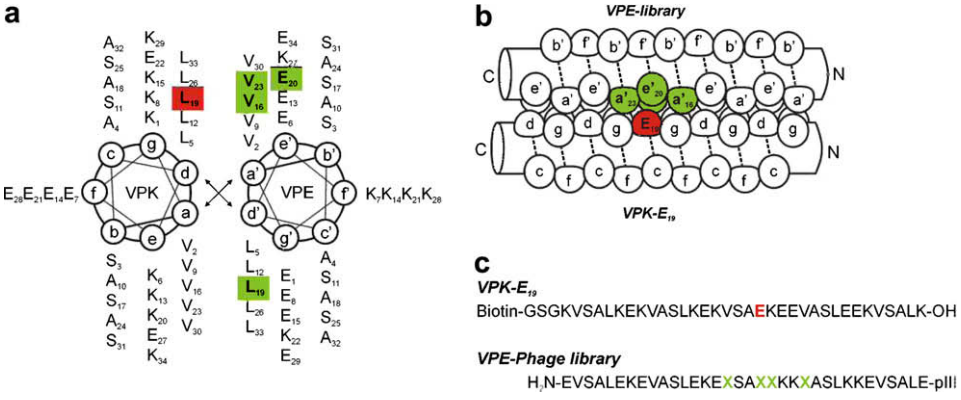

Figure 1. Helical wheel presentation of the parallel VPK-VPE heterodimer (a) and schematic side view of VPK-E_{19} and the VPE-library (b). Glu_{19} in VPK-E_{19} is highlighted in red, while the four randomized amino acid positions in VPE which directly interact with Glu_{19} are highlighted in green. (c) Amino acid sequences of VPK-E_{19} and the VPE-phage library.