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Previews

Grafting Miniature DNA Binding Proteins

Miniature proteins serve as leads for biological and medicinal applications by positioning all amino acids necessary for biomolecular recognition on a compact protein structure. Protein grafting was recently used to create miniature helical proteins with high DNA binding affinity and specificity.

Helical protein structures mediate a variety of important biomolecular interactions, including protein-protein, protein-DNA, and protein-RNA interactions. The ability to design and control helical proteins' structure possesses potential for the development of biological tools to probe protein function in vivo and pharmaceutical drugs targeting helical proteins. Designed helical proteins can also feature functions not available in natural molecules, such as increased DNA binding specificity or DNA cleavage activity.

Multiple strategies aimed at designing helical proteins have utilized helix-stabilizing elements that bypass the energetic penalty required to nucleate the first helical turn in a disordered peptide. In protein grafting or surface grafting, helical structures involved in biomolecular interactions have been stabilized by positioning amino acids critical for molecular recognition onto small helical protein scaffolds [1–3]. For example, the polyproline peptide from avian pancreatic polypeptide (aPP) maintains helical content in its carboxy-terminal region via hydrophobic interactions with an amino-terminal polyproline helix [4]. The stable helical motif in aPP has been exploited as a scaffold for creating helical miniature proteins [1, 2, 5–8]. In a recent example, the DNA binding element of the Q50K *engrailed* homeodomain protein was grafted onto the solvent-exposed surface of aPP to create a monomeric miniature protein capable of recognizing target DNA [9].

Protein grafting has been performed also with other helical scaffolds, including the GCN4 leucine zipper [10], the F-actin bundling protein villin [11], the Zif268 zinc finger 2 [12], and the bee venom peptide apamin [13, 14]. In the case of apamin, helical content is maintained in its carboxy-terminal region via a disulfide-stabilized amino-terminal loop [15]. The S-peptide from RNase A was grafted onto the helical apamin scaffold to create a helical S-peptide. By incubating the helical S-peptide with the carboxy-terminal catalytic domain of RNase A, enzymatic activity was reconstituted [13]. In a second example, the amino-terminus of the rhodanese protein was grafted onto apamin, and helical rhodanese protein was analyzed for binding to its cpn60 chaperone protein [14]. In these cases, protein grafting generated helical peptides for use in probing the role of helices in protein folding and function.

An ideal application of miniature proteins is toward DNA binding. DNA binding proteins contain various structural elements to interact with DNA, but the most prevalent include protein α helices [16]. Protein grafting using the helical aPP scaffold has been exploited in constructing miniature DNA binding proteins with high affinity and specificity [1, 2, 9]. Miniature proteins that interact sequence specifically with DNA have potential application toward development of artificial transcription factors for targeted inhibition or activation of gene expression [17].

In this issue of *Chemistry & Biology*, Turner et al. exploited the helix-stabilizing apamin in creating a miniature DNA binding protein [18]. By grafting the MyoD basic helix-turn-helix DNA binding element onto the apamin scaffold, an apamin/MyoD hybrid (apaMyoD) was created that bound its target DNA site with high affinity by preorganizing the DNA binding helix.

To initially probe the helical content of the apaMyoD fusion, circular dichroism (CD) studies were used. As expected, the apamin amino-terminal loop stabilized helical structure in the carboxy-terminal MyoD DNA binding peptide, increasing helical content from 7%, or 4 amino acids, in the native MyoD to 27%, or 19 amino acids, in apaMyoD. In this case, the disulfide bonds in amino-terminal loop nucleated helical structure over approximately 10 amino acid residues in the DNA binding helix. Importantly, helical content was diminished under reducing conditions where the disulfide bonds were disrupted, indicating that disulfide bonds are required to preorganize the MyoD helix. The increase in helical content of apaMyoD was comparable to the protein grafting strategies employing aPP, which stabilized helical content in up to 21 amino acids [2, 5].

To explore the DNA binding ability of the apaMyoD fusion, CD and fluorescence anisotropy studies were employed. The presence of apamin increased the melting temperature of the apaMyoD dimer/DNA complex by 13° compared with the native MyoD dimer, as assessed with CD. Fluorescence anisotropy studies indicated that the presence of apamin stabilized the apaMyoD dimer/DNA complex by -1.7 kcal•mole^{-1} relative to native MyoD. As expected, the increased DNA binding affinity of apaMyoD was lost under reducing conditions where disulfide bonds were disrupted.

Turner et al. were also interested in probing the selectivity of DNA binding by apaMyoD, or the ability to discriminate between target site DNA sequence and nontarget site DNA sequences. Despite the apparent lack of selectivity of native MyoD toward binding target site versus nontarget site DNA ($\Delta\Delta G = -0.07$ kcal•mole^{-1}), the apaMyoD chimera demonstrated higher affinity for its consensus site than for a random sequence of DNA ($\Delta\Delta G = -1.42$ kcal•mole^{-1}). Therefore, apaMyoD not only allowed for high affinity binding to target DNA sites in an disulfide-dependent manner, but it also afforded a specific interaction not seen in the native MyoD protein. Although other strategies have been employed to increase DNA target site selectivity in miniature proteins,

including incorporation of unnatural amino acids [19], protein grafting has proven successful in imparting DNA binding selectivity in naturally nonselective proteins, as exemplified by Turner et al. and others [1, 2, 9, 18]. Certainly, a critical requirement for future application of miniature DNA binding peptides is high selectivity for target DNA sites.

The recent advancements in the creation of miniature DNA binding protein hold promise for probing protein folding or for use as artificial transcription factors. To provide even more control, the future generations of miniature DNA binding proteins might incorporate small molecule control of DNA binding or couple with additional activities, like DNA cleavage. For example, fusion of the DNA binding element of the *engrailed* homeodomain peptide with EF hand Ca loop binding domain resulted in the creation of DNA nuclease [20]. In this example, the helix stabilization, DNA binding, and DNA cleavage were dependent on the addition of lanthanide metal, providing an additional level of control for potential applications. In addition, the lanthanide metal mediated DNA cleavage, providing useful activity in a miniature DNA binding protein. As a necessary step toward applications in vivo, like artificial transcription factors, miniature DNA binding proteins must be tested for activity in mammalian cells. Although further development is required, miniature DNA binding proteins represent an exciting tool for exploring various biological and medicinal problems.

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Don't Classify Polyketide Synthases

Polyketide synthases are intensively studied as metabolite factories generating diverse biologically active natural products. Contrary to their current classification as different "types," there is now a growing body of evidence illustrating that nature realized limitless transitional stages during evolution.

Polyketides are a remarkable class of natural products. In addition to an enormous range of functional and structural diversity, they exhibit a wide range of biological activities, applied in the agrochemistry and pharmaceutical industries, which triggered research aimed at a molecular understanding of their biosynthesis. Since the cloning of the first sets of polyketide synthases (PKS) from streptomycetes, numerous different "types" of PKS genes have been identified from a variety of biological sources, mostly bacteria, fungi, and plants, but recently also from protists [1–4]. Although investigations revealed that PKSs show some striking similarities to fatty acid synthase, a more or less systematic approach has been taken to classify the different types of systems based on their source or products as well as biochemical or genetic data.

Recently, it has become evident that transition states between differently classified biosynthetic systems exist. Nature's ingenuity for producing natural products is obviously not restricted to "classes" of natural product biosynthetic systems. For example, the "parallel and convergent universes of PKS and nonribosomal peptide synthetases" (NRPS) [5] were only recognized a few years ago after the first true hybrid PKS/NRPS systems with translationally fused PKS and NRPS modules were