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Nativelike Structure in Designed Four α-Helix Bundles Driven by Buried Polar Interactions

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It has proven to be relatively straightforward to design peptides that assemble into α-helical bundles which have strong secondary structure but lack tertiary specificity. The achievement of nativelike tertiary structure continues to be challenging, whether approached using computationally packed protein cores[1,2] or by binary patterned combinatorial peptide libraries.[3,4] There has been some indication that buried polar interactions can promote nativelike tertiary structure by imposing orientational specificity on helices: Efimov determined that the great majority of helices in the PDB contain at least one such interaction,[5] and Gratkowski et al. have shown these interactions can drive membrane helical assembly.[6] Here we demonstrate that such buried polar interactions, together with correct placement of hydrophobic and hydrophilic side chains, are sufficient to induce nativelike structure in helical bundle proteins.

We have previously reported the design and structure of the (α-*SS*-α)$_2$ apoprotein BB (all protein sequences and scaffolds are in Supporting Information Table 1).[7] In this protein each individual dihelical monomer is well-defined and in an anti topology, while the intermonomer packing interface is molten globular by NMR and is unusually poorly packed in X-ray structures.[8] A core histidine residue on each helix, H24, was found to hydrogen bond to the same residue on its parallel partner within each monomer (see Figure 1A). Notably, the molten-globular interface was the one lacking a hydrogen-bonding interaction. These data suggest that this buried hydrogen bond alone is sufficient to specify a unique structure.

To test this hypothesis, we have synthesized a variant of BB with the sterically conservative mutation of both histidines to phenylalanine and find that this abolishes tertiary specificity (see Figure 1B) while secondary structure is retained (see Supporting Information). Thus, despite the higher degree of intercalation between helices on the same monomer, we demonstrate that this single hydrogen bond in each helix is necessary to lift the protein out of the molten globular state.

We recently reported the structure-based redesign of BB into a structured diheme (α-*SS*-α)$_2$ bundle denoted HP-1.[9] HP-1 was redesigned to preorganize the apoprotein for heme binding by moving the histidine ligands out of the core. In contrast to BB, HP-1 is a molten globular apoprotein which becomes structurally specific upon binding ferric heme. The helical orientation model explains this: in the absence of heme, the apoprotein helices are not compelled to have a unique rotational orientation. The bis-histidine ligation of two molecules of heme, utilizing one histidine on each helix, fixes the orientation of the entire molecule.

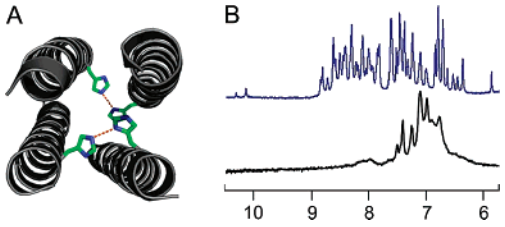

Figure 1. (A) Buried Nδ−Nϵ H-bonding interactions in the binary-patterned four α-helix protein BB. H-bonds are between the same residue, histidine 24, on each identical helix. While the pairs of helices that H-bond with each other have unique tertiary structure, the two dihelical monomers are highly mobile with respect to each other on the NMR time scale. (B) 1-Dimensional ^{1}H NMR spectra of BB (top) and BB-F10F24 (bottom). Despite the sterically conservative nature of this mutation, removal of the single internal hydrogen bond to each helix creates a constitutive molten globule.

We have further examined this using a redesigned diheme four α-helix bundle protein, HP-7. This protein, the design and NMR resonance assignments of which will be reported fully elsewhere, takes the form of a homodimer of helix−loop−helix peptides with the two peptide loops connected by a disulfide linkage, a scaffold that we term a "candelabra" motif. Bishistidine heme binding occurs between protein monomers, connecting the *N*-terminal helical pair using the two histidines at the 7 position and the C-terminal pair using the two histidines at the 42 position. Despite the fact that the four helices have identical sequences, the asymmetric nature of this motif causes each helix to have unique chemical shifts, allowing us to distinguish interactions occurring at the two heme binding sites.

As we reported for HP-1,[9] the apoprotein HP-7 is a molten globule. This is identified in ^{15}N-HSQC spectra by the absence of chemical shift dispersion; all amide resonances display their random coil chemical shifts (see Figure 2A) while the protein retains helical secondary structure as detected by CD (see Supporting Information). Addition of 1 equiv of the symmetric heme Fe^{+3}-protoporphyrin III to the bundle causes two of the helices to become uniquely structured. This is apparent from the appearance of disperse chemical shifts corresponding to those assigned to the helices containing histidine-7 in the diheme protein, with the resonances corresponding to the second pair of helices retaining their random coil values (Figure 2B). Addition of a second equivalent induces nativelike structure in the remaining two helices (Figure 2C). These assignments were verified by NMR analysis of the heme complexes of HP-7 H7F and HP-7 H42F (data not shown).

Despite their substantially different cofactor structures, similar results are seen in the complexes of HP-7 with either heme A or

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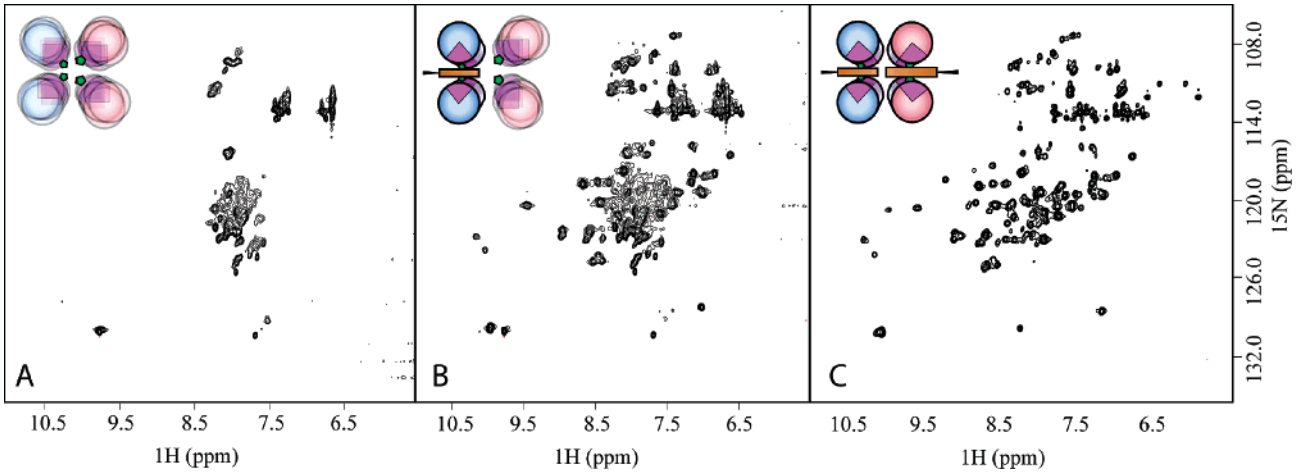

Figure 2. Stepwise helical orientation upon heme binding to HP-7: 750 MHz ^{15}N-HSQC spectra of 350 μM HP7 dimer in 25 mM KH_2PO_4, pH 6.6 at 18 °C. (A) The apoprotein is a molten globule. (B) One molecule of the symmetric heme Fe^{+3}-protoporphyrin III per bundle structures two of the four helices. (C) Two hemes per bundle induces complete structuring; the variation in peak intensities is due to the presence of two paramagnetic ferric hemes.

Zn^{2+}-bacteriochlorophyllide (BChl), with the formation of a number of disperse resonances corresponding to the distinctly different structuring of two helices for the bishistidine ligation of iron in heme A and one helix in the single histidine ligation of zinc in bacteriochlorophyllide (see Supporting Information.) The complex with Mn^{2+}-BChl, which prefers carboxylate ligands,[10] is a molten globule owing to the misalignment of the helical patterning caused by this coordination. It is remarkable that tertiary structural specificity is conferred on a helix from single H-bonds between histidines (apo-BB, Figure 1) or from single coordination between histidine and metal in a porphyrin or chlorin. Moreover, the ability of this family of simple four helix bundles to accommodate a variety of metal porphyrin and chlorin structures while adopting complementary, singular structures around them evinces both a considerable structural plasticity and a natural tendency to fold into a uniquely structured product.

This work shows that complementarity in hydrophobic core packing is not an obligatory design element in assuring tertiary specificity in proteins. Our data support a model in which interactions between pairs of buried polar residues can be sufficient to specify unique helical orientation resulting in structural and spectroscopic properties typical of natural proteins. Moreover we show that correct binary patterning combined with helix alignment established by residues as ligands to cofactors arrives at uniquely structured solutions a significant fraction of the time; explicit computational selection and placement of side chains is not necessary to create structured cofactor-binding proteins. The observed "jigsaw puzzle"-like core packing observed in natural proteins may be a consequence of natural selection for many concurrent functions of which structural specificity is just one.[11,12]

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Supporting Information Available: Peptide sequences, CD spectra, ^{15}N-HSQC spectra of the complexes of HP-7 with Heme A, Zn^{2+}-BChl and Mn^{2+}-BChl. ^{1}H NMR spectrum of the Fe^{2+}-proptoporphyrin III complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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