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Sequence-specific resonance assignments for a designed four-α-helix bundle protein

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Biological context

We have undertaken to explore the basic structural features necessary to support specific biological function of electron transfer proteins by designing minimalist protein models of the photosynthetic reaction center, the bc1 complex, iron–sulfur proteins and others with small peptides that self-assemble to a four-α-helix-bundle architecture and bind the necessary prosthetic groups (Robertson et al., 1994). As designed, the prototype maquette (H10H24) folds to a four-α-helical structure, binds hemes with the expected stoichiometry and affinity, and mimics the redox properties of the hemes in the cytochrome bc1 complex (Robertson et al., 1994). Successful as this prototype design was, the molecule still lacked a unique solution conformation as defined by NMR spectroscopy and probably exists as a mixture of different conformers that are interconverting slower than the microsecond timescale. This is a characteristic problem of most de novo protein designs (Betz et al., 1993) and hampers meaningful characterization of the protein functional properties. In order to improve upon the conformational specificity, two of the heptad *d*-positions at the designed helix packing interface were targeted for substitution. Single substitutions, H10H24-L6I and H10H24-L13F, resulted in molecules that exist primarily as single conformers in solution while the double variant H10H24-L6I,L13F shows a single set of NMR resonances with narrow linewidths and chemical shift dispersion characteristic of a native protein. The latter protein will now serve as the parent molecule for a series of structural and functional investigations of itself and related proteins. This note presents the resonance assignment of the twofold symmetric molecule, provides a strong reference point for beginning to understand the origin of chemical shift perturbations brought by the attainment or loss of native-like structure, and describes our progress toward the solution structure determination of H10H24-L6I,L13F by NMR spectroscopy.

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Methods and results

H10H24-L6I,L13F [gsCGGGEIWKLHEEFLKKFEE LLKLHEERLKKL]^2 was cloned and expressed as a thioredoxin fusion protein. The peptide is released from the fusion protein with thrombin and the N-terminal cysteines are air oxidized to form a pair of linked helices. Protein solutions were prepared in a 20 mM sodium phosphate pH 6.60, 50 mM potassium chloride, 0.05 mM sodium azide, 92% H_2O/8% D_2O. All experiments were performed on a Varian Inova 500, Inova 600, or Inova 750 spectrometer.

Main-chain C^α, N, H^N and side chain C^β resonances were assigned using HNCACB (Wittekind and Mueller, 1993) and CBCA(CO)NNH (Grzesiek and Bax, 1992) to establish segments of sequential connectivity. Main-chain H^α and C' resonance assignments were then completed using CBCACO(CA)HA (Kay, 1993), HNCO (Kay et al., 1990) and ^{15}N-edited TOCSY (Zhang et al., 1994). Ambiguous connectivity was resolved using the ^{15}N-edited TOCSY and

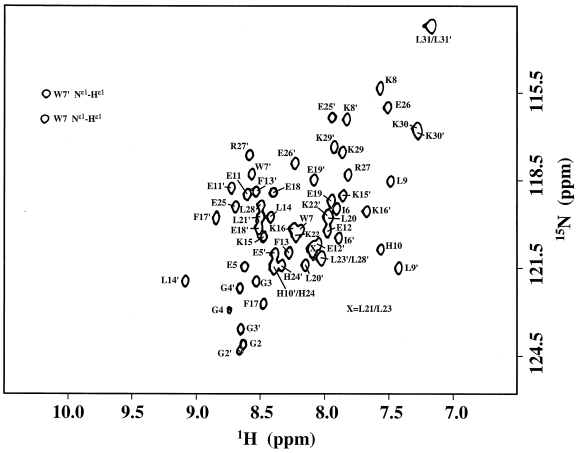

Figure 1. ^{15}N-HSQC spectrum of 2.0 mM uniform ^{15}N-labeled H10H24-L6I,L13F at 600 MHz ^{1}H frequency showing two sets of correlations. The H^N correlations for G2, G2′, G3, G3′, G4, G4′, L31, L31′, W7-$H^{\epsilon 1}$ and W7′-$H^{\epsilon 1}$ are aliased.

NOESY spectra to identify sequential H^N-H^N and H^α-H^N NOEs characteristic of helical proteins. Finally, selectively ^{15}N-glutamate and ^{15}N-lysine labeled proteins provided unambiguous checks for these amino acids. The aliphatic side-chain carbon and proton assignments were made with CC(CO)NNH TOCSY (Montelione et al., 1992) and HCCH-TOCSY (Bax et al., 1990). Leucine δ-methyl prochirality was established using partially ^{13}C-labeled protein and ^{13}C-HSQC spectroscopy (Neri et al., 1989).

Extent of assignments and data deposition

The main chain C^α, C′, N, H^α, H^N and side-chain C^β resonance assignments are complete for the Cys^1-Leu^{31} and Cys^{1}'-Leu$^{31'}$ chains with the exception of the N and H^N resonances for Cys^1 and Cys$^{1'}$ (see Figure 1 and Supplementary Table 1). Side-chain resonance assignments for leucine and isoleucine residues are complete and assignments for the glutamate, arginine, and lysine residues are ∼85% complete. Glu^5-Lys^{30} and Glu$^{5'}$-Lys$^{30'}$ show C^α and C′ shifts downfield of random coil values and the C^β and H^α shifts upfield of random coil values characteristic of α-helix secondary structure. The designed linker region Cys^1-Gly^4 and Cys$^{1'}$-Gly$^{4'}$ show shifts expected for an extended conformation. The assigned shifts are compiled in Suplementary Table 1 and deposited under accession number 4065 in the BioMagResBank database.

Our current working structural model of the bundle is a four-α-helix protein comprised of two asymmetric di-α-helices that are related by a twofold symmetry axis. The magnetic asymmetric unit is the di-α-helix and the asymmetry most likely arises from side-chain packing. A four-α-helix bundle stoichiometry (calculated MW 15.6 kDa) was established using sedimentation equilibrium (∼17 kDa), size-exclusion chromatography (∼18 kDa), and self-diffusion (∼17 kDa) measurements.

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References

Bax, A., Clore, G.M. and Gronenborn, A.M. (1990) *J. Magn. Reson.*, **88**, 425–431.

Betz, S.F., Raleigh, D.P. and DeGrado, W.F. (1993) *Curr. Opin. Struct. Biol.*, **3**, 601–610.

Grzesiek, S. and Bax, A. (1992) *J. Am. Chem. Soc.*, **114**, 6291–6293.

Kay, L.E. (1993) *J. Am. Chem. Soc.*, **115**, 2055–2057.

Kay, L.E., Ikura, M., Tschudin, R. and Bax, A. (1990) *J. Magn. Reson.*, **89**, 496–514.

Montelione, G.T., Lyons, B.A., Emerson, S.D. and Tashiro, M. (1992) *J. Am. Chem. Soc.*, **114**, 10974–10975.

Neri, D., Szyperski, T., Otting, G., Senn, H. and Wüthrich, K. (1989) *Biochemistry*, **28**, 7510–7516.

Robertson, D.E., Farid, R.S., Moser, C.C., Urbauer, J.L., Mulholland, S.E., Pidikiti, R., Lear, J.D., Wand, A.J., DeGrado, W.F. and Dutton, P.L. (1994) *Nature*, **368**, 425–431.

Wittekind, M. and Mueller, L. (1993) *J. Magn. Reson.*, **B101**, 201–205.

Zhang O., Kay, L.E., Olivier, J.P. and Forman-Kay, J.D. (1994) *J. Biomol. NMR*, **4**, 845–858.