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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of S-824, a *de novo* four-helix bundle from a designed combinatorial library

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Biological context

Protein design has emerged as an important tool for elucidating the relationship between amino acid sequence and three-dimensional structure. Previously, we described the construction of combinatorial libraries of *de novo* proteins (Kamtekar et al., 1993; West et al., 1999; Wei et al., 2003). These libraries were devised using binary patterning of polar and nonpolar amino acids as the major design constraint. In a library of binary-patterned proteins, each sequence position is designed explicitly to be either polar or nonpolar; however, the precise identities of the amino acids are varied extensively. Polar amino acids can be Lys, His, Glu, Gln, Asp or Asn, while nonpolar amino acids can be Met, Leu, Ile, Val or Phe. These mixtures of polar and nonpolar amino acids are encoded by the degenerate DNA codons VAN and NTN, respectively (V = A, G or C; N = A, G, C or T).

The combinatorial underpinnings of the binary code strategy preclude explicit design of particular side-chains at specified positions. Therefore packing interactions cannot be specified *a priori*. To assess whether the binary code strategy can nonetheless produce well-folded *de novo* proteins, we constructed a library using a scaffold of 102 residues designed to fold into four-helix bundles (Wei et al., 2003). Five proteins from this library were characterized, and four of them were shown to possess features consistent with the formation of stable and/or native-like structures (Wei et al., 2003). One of these four proteins, S-824, was then chosen for more detailed structural and dynamic studies. Here we report the ^{1}H, ^{15}N and ^{13}C resonance assignments of protein S-824.

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Methods and experiments

The 102-residue sequence of the *de novo* protein S-824 is MYGKLNDLLEDLQEVLKNLHKNWH GGKDNLHDVDNHLQNVIEDIHDFMQGGGSGG KLQEMMKEFQQVLDELN NHLQGGKHTVHHIE QNIKEIFHHLEELVHR. This sequence is from a 2nd generation library of binary code proteins described by Wei et al. (2003). The protein was expressed in *E. coli* BL21(DE3) as described previously (Kamtekar et al., 1993; Wei et al., 2003). Uniformly ^{15}N and ^{15}N/^{13}C labeled samples were prepared by growing bacteria in M9 minimal media supplemented with 1 g l^{-1} ^{15}N-NH_4Cl and 2 g l^{-1} ^{13}C-glucose. Protein was purified as described previously (Kamtekar et al., 1993; Johnson et al., 1994; Wei et al., 2003). Sample concentration was ∼1.5 mM in 50 mM HAc-NaAc buffer, pH 4.0 in 92% H_2O/8% D_2O. All NMR experiments were performed on a Varian Inova 600 NMR spectrometer equipped with a triple resonance probe and pulse field gradients. The ^{1}H, ^{13}C and ^{15}N chemical shifts were referenced to DOH using indirect methods (Wishart et al., 1995, 1998). Spectra were processed and analyzed with Felix 97 software (MSI, San Diego, CA). The NMR exper-

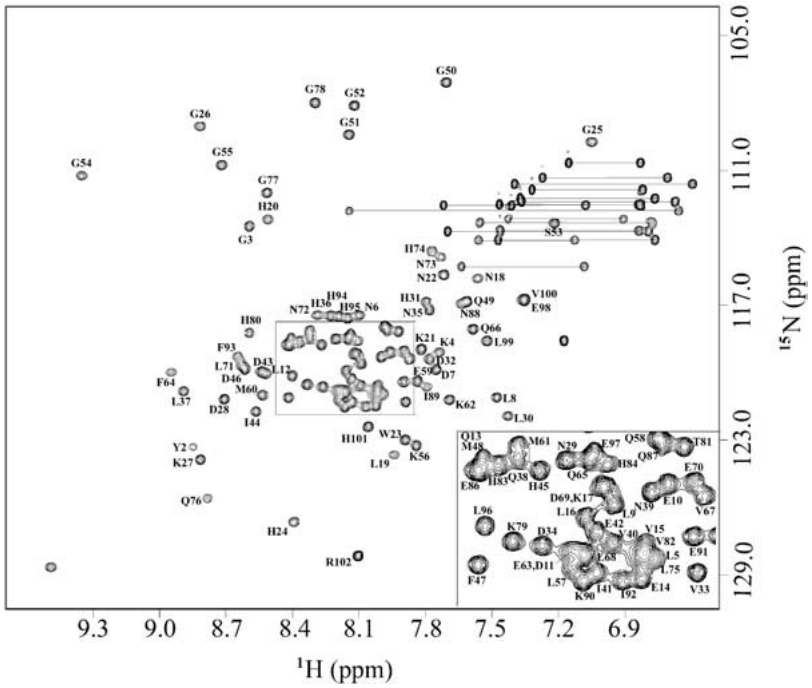

Figure 1. ^{1}H- ^{15}N HSQC spectrum of S-824 recorded at 298 K with the assignments indicated. The inset (the lower right) is an expanded view of the more crowded region in the spectrum.

iments preformed included ^{1}H-^{15}N HSQC, ^{1}H-^{13}C HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH, (H)CC(CO)NH for backbone and H(C)CH-TOCSY, (H)CCH-TOCSY, and ^{15}N edited TOCSY for side chain assignments. Assignments were further confirmed by ^{15}N edited NOESY and ^{13}C edited NOESY.

Extent of assignments and data deposition

The backbone ^{1}H, ^{15}N and ^{13}C resonance assignments of S-824 are virtually complete. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum and backbone assignments of S-824. The 3D (H)CC(CO)NH experiment was very helpful as it allowed the unambiguous identification of individual amino acid spin systems in the extensively overlapped spectrum due to the predominantly (>80%) helical structure of the protein. In summary, backbone and side chain assignments were made to following extent: 100% of backbone ^{15}N, ^{1}H$^{\alpha}$, ^{13}C$^{\alpha}$, ^{1}H^{N} , ^{13}C$^{\beta}$ and ^{13}C$'$ resonances were assigned; 99% of H$^{\gamma}$, 90% of H$^{\delta}$ and 85% of H$^{\epsilon}$ resonances were assigned. The side chain resonances are virtually complete with over 95% assigned. The chemical shifts of S-824 have been deposited in the BioMagResBank under the accession number BMRB-5687.

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