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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of an affibody-target complex

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Biological context

Affibodies are small proteins with specific binding properties that have been engineered to obtain an alternative to antibodies. They have the scaffold of the 58 residue Z domain, derived from the B domain of Staphylococcal protein A (SPA), and their small size and high stability give several advantages compared to immunoglobulins. Affibodies are selected from phage-display libraries in which 13 solvent accessible residues on the Z domain have been subjected to combinatorial mutagenesis. The $Z_{SPA\text{-}1}$ affibody was selected for affinity to SPA and binds to the parental Z domain with a dissociation constant $K_d = 6$ μM (Eklund et al., 2002).

We report here essentially complete ^{1}H, ^{13}C and ^{15}N assignments of the 1:1 complex formed by the Z domain and the $Z_{SPA\text{-}1}$ affibody. The ^{15}N-HSQC spectrum of the isolated $Z_{SPA\text{-}1}$ affibody shows poor dispersion and line shape properties, indicating that it is not fully folded. However, the corresponding spectrum recorded on the $Z_{SPA\text{-}1}$ affibody in complex with the Z domain shows a significant improvement of resonance line shapes and dispersion. These results suggest a mechanism of coupled binding and folding of $Z_{SPA\text{-}1}$. Further NMR studies and structure determination of this complex can therefore be expected to aid improvements of binding protein libraries and selection conditions, and also contribute to the understanding of protein-protein interactions in general.

Methods and results

The $Z_{SPA\text{-}1}$ affibody and the Z domain were overexpressed separately in *E. coli* BL21 DE3 cells using the plasmid pET28a(+). The cells were cultured in either Luria broth medium or $^{15}NH_4SO_4$ (2 g l^{-1}) and/or ^{13}C-glucose (3 g l^{-1}) enriched minimal medium for isotopic labeling. Transformed cells were grown to OD_{600} of 0.6–1.0, induced with 1 mM IPTG and incubated over night at 17 °C. The cell pellets were suspended in TST buffer (25 mM TrisHCl pH 8.0, 1 mM EDTA, 200 mM NaCl, 0.05% w/v Tween 20) and lysed. The proteins were purified by affinity chromatography, using IgG (Z) or protein A Sepharose ($Z_{SPA\text{-}1}$), followed by gel filtration on a HiLoad Superdex 30 16/60 column using the Äkta explorer system (all equipment from Amersham Biosciences). The affinity columns were pre-equilibrated with TST and washed with TST and 5 mM NH_4Ac at pH 5.0 (only for the Z domain) after sample application. The proteins were eluted with 0.2 M HAc at pH 3.3 (Z) or 0.5 M HAc at pH 2.8 ($Z_{SPA\text{-}1}$). The buffer was changed to 20 mM NaP_i, 150 mM NaCl (pH 7.2) before gel filtration and the eluted fractions were dialyzed to 50 mM NH_4Ac (pH 7.2), lyophilised and dissolved in NMR buffer. A typical NMR sample of the Z:$Z_{SPA\text{-}1}$ complex contained 1.5 to 2.0 mM labeled Z (or $Z_{SPA\text{-}1}$) and 10 to 25% excess of unlabeled $Z_{SPA\text{-}1}$ (or Z) in 20 mM KP_i, 0.01% NaN_3, and 10% or 100% D_2O at pH 5.6 (uncorrected reading), except for samples with ^{15}N-labeled Z and unlabeled $Z_{SPA\text{-}1}$, for which the pH was 6.8.

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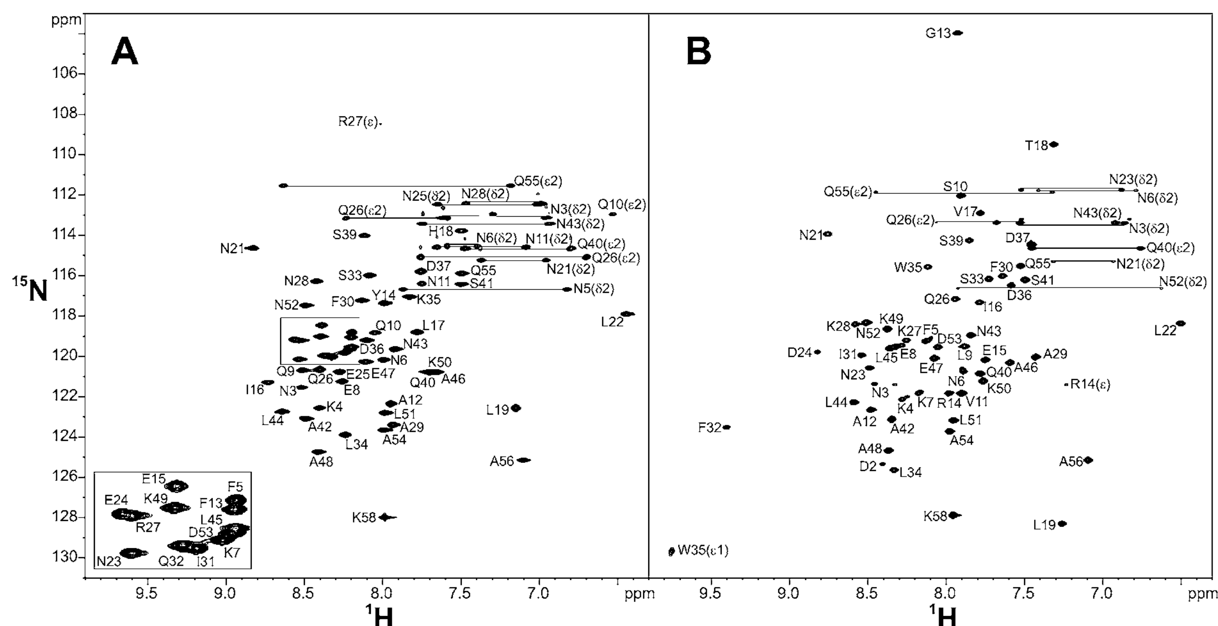

Figure 1. Assigned ^{15}N-HSQC spectra of the Z:Z$_{SPA-1}$ complex. (A) ^{15}N-labeled Z domain with unlabeled Z$_{SPA-1}$. (B) ^{15}N,^{13}C-labeled Z$_{SPA-1}$ affibody with unlabeled Z. Sidechain amide resonances are connected with lines. Resonances of Arg H$^{\varepsilon}$ appear as folded peaks.

NMR data were acquired at 30 °C on Bruker Avance 500 MHz and 600 MHz spectrometers and on a Varian Inova 800 MHz spectrometer equipped with 5 mm triple resonance (^{1}H, ^{13}C, ^{15}N) probes. Backbone and side chain assignments were obtained using CBCA(CO)NH, CBCANH, HNCO, HN(CA)CO, HCCH-COSY and HCCH-TOCSY (Cavanagh et al., 1996). The methyl groups of Val and Leu side chains were stereospecifically assigned using constant time ^{13}C-HSQC experiments recorded on 10% ^{13}C-labeled samples (Neri et al., 1989). ^{15}N-TOCSY-HSQC and ^{15}N-NOESY-HSQC experiments with short mixing times (28.8 ms, and 30.0 or 60.0 ms, respectively) and an HNHB experiment (for Z) were used to obtain χ_1 dihedral angles and stereo specific assignments of H$^{\beta}$ protons. Proton resonances in aromatic side chains were assigned based on 2D ^{13}C-filtered COSY experiments (Vuister et al., 1994). All NMR data were processed using XWIN-NMR (Bruker) or NMRPipe (Delaglio et al., 1995) and analysed using Ansig for Windows (Helgstrand et al., 2000).

Extent of assignment and data deposition

The backbone resonances were completely assigned, except for C′ of Asp37 in the Z$_{SPA-1}$ affibody and C′ and NH of N-terminal Val1 in the Z-domain. All resonances in the ^{15}N-HSQC spectra (Figure 1) were identified, including those of Arg H$^{\varepsilon}$, Trp H$^{\varepsilon 1}$ and all amides of Gln and Asn side chains. Altogether were 99.5% of all aliphatic, aromatic and backbone protons assigned. 30 out of 115 χ_1-dihedrals (and the corresponding stereochemistry of 28 pairs of H$^{\beta}$ protons) could be determined. All methyl groups of the Leu and Val residues in the Z-domain could be stereo-specifically assigned. Due to expression problems, this could not be done for the Z$_{SPA-1}$ affibody. The chemical shift assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5397.

Acknowledgements

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