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Letter to the Editor: Triple resonance-based assignment for Abl SH(32) and its complex with a consolidated ligand

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Biological context

The proto-oncogene *abl* product is involved in cellular signal transduction processes and cell-cycle regulation (Wang, 1993; Pendergast, 1996). This protein has several individually folded domains. Its N-terminal region contains, sequentially, a Src homology domain 3 (SH3), an SH2, and a functional kinase domain (SH1), which are similar to those of the Src family members. Abl also has a long C-terminal domain markedly distinguishing the Abl family from the Src family. Chromosomal translocation of part of the *Bcr* gene on chromosome 22 to the *abl* gene on chromosome 9 produces the Philadelphia chromosome. The resulting fusion protein is responsible for certain types of human leukemia. Experiments also suggest that the Abl SH3 and SH2 domains mediate its signal transduction interactively (Mayer and Baltimore, 1994). The SH3 of Abl appears to suppress the intrinsic transforming ability, while its SH2 is absolutely required for expression of the transforming activity of activated Abl. The SH3 and SH2 domains seem not to directly interact with each other in Abl in solution (Gosser et al., 1995). However, topological folding of these two closely located domains may still strongly affect the formation of the regulatory complex, and thus its function. The sequence-specific NMR assignment of the 156-residue Abl SH(32) dual domain system, and of its complex with a consolidated ligand (Cowburn et al., 1995), is presented. These assignments provide a basis for further structural perturbation studies, and dynamics analyses. Further structural information can provide insight for design of more tightly bound consolidated ligands, which may have potential therapeutic value by blocking the transformation activity of oncoprotein Abl.

Methods and results

The sequence of Abl-SH(32) studied here, from residue L65 to R220 (GemBank g125135), is as follows: gspggsLFVALYDFVASGDNTLSITKGEKL RVLGYNHNGEWAEAQTKNGQGWVPSNYITPVN SLEKHSWYHGPVSRNAAEYLLSSGINGSFLVRE SESSPGQRSISLRYEGRVYHYRINTASDGKLYVR SSESRFNTLAELVHHHSTVADGLITTLHYPAPKR gihrd, where the lower case letters represent those amino acids introduced by the expression system used. A mutation at residue C101A was introduced into the cDNA with PCR to increase NMR sample stability. Uniformly ^{15}N- and ^{13}C,^{15}N-labeled SH(32) were over-expressed as GST-fusion proteins in *E. coli* DH5α cells grown in M9 medium at 37 °C. $^{15}NH_4Cl$ and ^{13}C-glucose were applied as sole nitrogen and carbon sources. SH(32) was released from GST by thrombin cleavage and further purified by gel filtration. The yield was 12 mg per liter of culture.

The consolidated ligand contains an Abl SH3 binding peptide PPAYAPPPVP (3BP), and an Abl SH2 binding peptide PVpYENV (2BP), whose C-termini are joined by G_6-Kamide through an amide bond between the lysyl side chain and the oligoglycyl linker (Cowburn et al., 1995). It was not isotope enriched.

All ^{15}N-labeled, and ^{13}C/^{15}N-labeled SH(32) and SH(32)/consolidated ligand complex samples were exchanged into a phosphate buffer (200 mM NaCl, 4.3 mM sodium phosphate, 2.7 mM KCl, 1.4 mM potassium phosphate, pH 7.2, containing 8% v/v D_2O, 2 mM EDTA-d_{12}, 2 mM DTT-d_{10}, and 0.02% w/v

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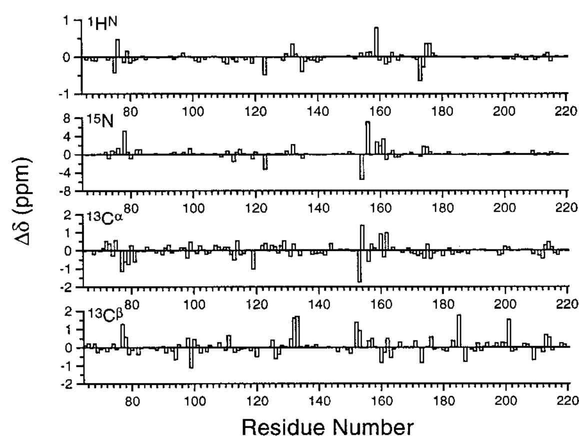

Figure 1. Summary of the chemical shift deviations of Abl SH(32) between ligand-bound and ligand-free forms. From top to bottom, the differences in $^1H^N$, ^{15}N, C^α and C^β, respectively, are plotted.

NaN_3). The consolidated ligand was added to SH(32) at a 1.15 to 1 ratio. The protein concentration was kept at 0.8 mM for all samples to reduce protein aggregation. Samples were 500 μL in volume.

A Bruker DMX 600 MHz spectrometer with a 5 mm probe was used to acquire the following spectra, recorded, at 308 K, with the indicated number of complex points in the indicated dimension: $^1H,^{15}N$-HSQC (2048 ($t_2,^1H$) × 256 ($t_1,^{15}N$), HNCA (1024 ($t_3,^1H$) × 50 ($t_2,^{13}C$) × 60 (t_1, ^{15}N)), HN(CO)CA (1024 ($t_3,^1H$) × 50 ($t_2,^{13}C$) × 50 ($t_1,^{15}N$)), CBCA(CO)NH (1024 ($t_3,^1H$) × 46 (t_2, ^{15}N) × 104 ($t_1,^{13}C$)), $^1H,^{15}N$-HSQC-TOCSY (1024 ($t_3,^1H$) × 68 ($t_2,^{15}N$) × 280 ($t_1,^1H$)), ^1H, ^{15}N-HSQC-NOESY (1024 ($t_3,^1H$) × 48 ($t_2,^{15}N$) × 512 ($t_1,^1H$)), and HCCH-TOCSY (512 ($t_3,^1H$) × 118 ($t_2,^{13}C$) × 100 ($t_1,^1H$)). H_2O suppression was carried out either with the WATERGATE method (Sklenar et al., 1993), or by selective on-resonance irradiation during inter-acquisition delay. All data was processed with XWINNMR (Bruker). In all cases, the chemical shifts of ^1H were referenced to external sodium 2,2-dimethyl-2-silapentane-5-sulfonate (Live et al., 1984). Indirect referencing was used for ^{13}C and ^{15}N (Live et al., 1984). The time domain data were zero-filled to the next higher power of 2.

Resonance assignment was carried out using the program XEASY (Bartels et al., 1995). All of the backbone H^N, N and C^α chemical shifts were assigned based on the combined use of 2D $^1H,^{15}N$-HSQC, 3D HNCA, and 3D HN(CO)CA spectra. A 3D CBCA(CO)NH experiment was used to obtain the C^β chemical shift information and to check the backbone assignment based on the C^α/C^β chemical shift pattern (Grzesiek and Bax, 1993). H^α chemical shifts were determined from 3D HSQC-TOCSY and 3D HSQC-NOESY spectra. Complete assignments of the CH_n of aliphatic side chains, and the H^β of aromatic side chains were obtained by 3D HCCH-TOCSY. Proline spin systems were identified in 3D HCCH-TOCSY spectra as well, starting from their C^α and C^β chemical shifts obtained from a CBCA(CO)NH spectrum. The $-NH_2$ groups of Asn and Gln were assigned sequence-specifically using $^1H,^{15}N$-HSQC, HN(CO)CA and HSQC-NOESY spectra.

Extent of assignments and data deposition

For SH(32) without ligand, 142 out of 150 backbone amide resonances were unambiguously assigned. Among those unassigned were Ser^{126}, Ser^{143}, Ser^{156}, Ser^{180}, Ser^{188}, Asp^{77}, Lys^{105}, and Leu^{122}. The C^α, H^α and the side-chain ^{13}C and ^1H chemical shifts of 126 aliphatic residues, including five prolines, were assigned. All 25 aromatic residues had their C^α, H^α, C^β and two H^β's assigned.

In the case of SH(32) bound to its consolidated ligand, 144 backbone amides were assigned. Compared with SH(32) without ligand, Asp^{77}, Lys^{105}, Ser^{126}, Ser^{143}, Ser^{156}, Ser^{180}, and Ser^{188} were identified for the complex. The amides of residues His^{95}, Ser^{121}, Arg^{134}, and Glu^{155}, however, became undetectable in the complex. About 7% of the side-chain resonances were unassigned as well.

The chemical shifts of ^1H, ^{15}N and ^{13}C resonances for Abl SH(32), and for SH(32) bound to a consolidated ligand with C-terminal to C-terminal linkage at pH = 7.2 and T = 308 K, have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession numbers 4251 and 4252.

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