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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of the SH3-SH2 domain pair from the human tyrosine kinase Lck

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Biological context

The lymphoid-specific cellular kinase (Lck) is a Src-related non-receptor protein tyrosine kinase essential for T-cell development and function. It is tightly associated with the cytoplasmic parts of the CD4 and CD8 receptors and catalyzes the initial phosphorylation of T-cell receptor components necessary for signal transduction and T-cell activation (Isakov and Biesinger, 2000). Lck exhibits a molecular architecture typical for the Src family of tyrosine kinases (Src, Blk, Fgr, Fyn, Hck, Lck, Lyn, Yes, Yrk): A myristylated N-terminal 'unique' domain is followed by the regulatory SH3 and SH2 domains and by the kinase domain containing the active site. The C-terminal region contains a regulatory tyrosine residue (Tyr505) which is bound to the SH2 domain in its phosphorylated form thereby reducing kinase activity. In addition, the SH3 and SH2 domains can negatively regulate kinase activity by forming intramolecular contacts that stabilize the catalytic domain in an inactive conformation. Release of these intramolecular regulatory constraints by dephosphorylation of Tyr505 or by the presence of SH2/SH3 competing ligands results in a catalytically active kinase. For full activation, dissociation of both regulatory domains from the kinase domain is required. Molecular dynamics simulations and site-directed mutagenesis experiments suggest a state-dependent coupled dynamics of the SH3-SH2 domain pair in which the SH3-SH2 connector appears to be an inducible 'snap lock' that clamps the SH2 and SH3 domains upon tail phosphorylation, but which allows flexibility when the tail is released (Young et al., 2001).

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Structural information on the regulatory domains of Lck is yet limited to the solution structure of the SH3 domain (Schweimer et al., 2002) and the crystal structure of the SH3-SH2 domain pair (Eck et al., 1994). The latter exhibits a rather unusual domain orientation that deviates strongly from those observed in other Src-kinases. This observation suggests that structure and dynamics of the regulatory domain pair might not only be state-dependent, but might also differ between various Src-kinases (Arold et al., 2001).

Structural studies of the Lck SH3-SH2 domain pair (LckSH32) in solution are thus expected to give further insight into the properties of the domain interface as well as the effects of SH2 or SH3-binding ligands on the structure and dynamics of the domain pair. As a first step towards addressing these questions, we assigned ^{1}H, ^{13}C and ^{15}N resonances from multidimensional heteronuclear NMR data.

Methods and results

The cDNA comprising the SH3 and SH2 domain of human Lck (aa 57-225, LckSH32) was cloned via PCR into the *NdeI* and *Bpu1102I* restriction sites of a pET11a vector (Novagen, Madison, USA) in order to produce tagless recombinant protein in *E. coli* BL21(DE3) cells. For isotope labeling according to the procedure of Marley et al. (2001), cells were first grown in 6 l unlabeled LB medium containing 100 μg ml^{-1} ampicillin at 37 °C up to an OD_{600} of 0.8. After centrifugation, the pellets were resuspended in 1.5 l medium containing $^{15}NH_4Cl$ and ^{13}C glucose as sole nitrogen and carbon source, respectively, and expression of the recombinant protein was induced by addition of IPTG to a final concentration of 0.8 mM. LckSH32

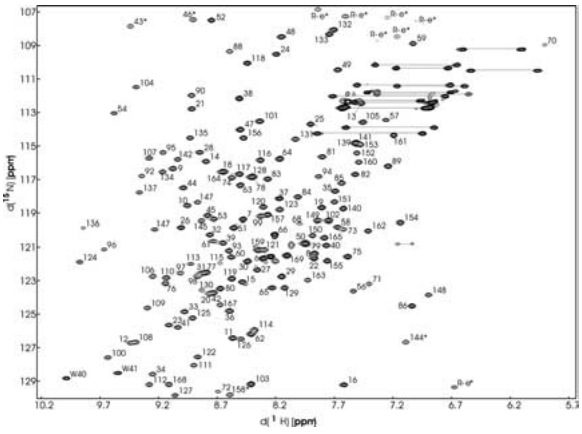

Figure 1. 700 MHz [^{15}N,^{1}H]-HSQC spectrum of ^{13}C/^{15}N labeled LckSH32 at 25 °C. Resonances are labeled with the corresponding sequence positions. Sidechain NH_2 resonances are connected with a line. W and R denotes sidechain resonances of arginines. Peaks with an asterisk are aliased along the ^{15}N dimension (the ^{15}N sweep width was 23.5 ppm).

was purified by affinity chromatography to phosphotyrosine residues immobilized on an Actigel-ALD column that was prepared according to the manufacturers guidelines (Sterogene Bioseparations Inc., Carlsbad, U.S.A.). The protein was eluted by addition of 1 M NaCl to the wash buffer (20 mM Tris/HCl, pH 7.5) and the correct mass of the LckSH32 was confirmed by ESI mass spectrometry using an unlabeled sample of LckSH32.

All NMR experiments were acquired on Bruker DRX 600 and Avance 700 NMR spectrometers at a temperature of 25 °C. The following 3D-NMR spectra were recorded for backbone and aliphatic resonance assignment: HNCO, HNCA, HNCACB, CBCA(CO)NH, H(C)CH-COSY, HBHA(CO)NH, HC(C)H-TOCSY, CCONH (Bax and Grzesiek, 1993; Sattler et al., 1999). The NMR data were processed using in house written software and was analyzed with NMRView 5.0.4 (Johnson and Blevins, 1994).

The backbone resonances were semiautomatically assigned with an in house written search algorithm using inter- and intraresidual C^{α} and C^{β} chemical shifts for sequential linking of amide resonances and amino acid type determination. Aliphatic sidechain carbon and proton resonances were assigned by analyzing the HBHA(CO)NH, H(C)CH-COSY, C(CO)NH and HC(C)H-TOCSY data. An assigned [^{15}N, ^{1}H] HSQC spectrum of LckSH32 is shown in Figure 1.

Extent of assignment and data deposition

The analysis of the triple resonance spectra allowed the identification and sequential assignment of 157 backbone amide resonances. No amide resonances could be found for H91 and for the aminoterminal region A1-D6 presumably either due to conformational exchange or to exchange with the solvent. For all 168 assignable residues the C^{α} and C^{β} chemical shifts could be assigned. The H^{α}, C^{α} and C^{β} assignment is complete for all observed residues with partial exception for the aminoterminal region A1-Q5. H^{β} chemical shifts could be be assigned to an extent of $> 95\%$ and the other aliphatic proton and carbon sidechain resonances could be assigned to an extent of $> 85\%$.

Comparison of the chemical shifts of the SH3 domain as part of LckSH32 with the shifts of the isolated SH3 domain (Schweimer et al., 2002) reveals only minor differences ($\Delta\delta(^{1}H) < 0.05$ and $\Delta\delta(13C)$, $\Delta\delta(^{15}N) < 0.5$ ppm) for residues 5–60 of LckSH3, while larger differences are observed for residues 61–63 located in the linker between SH3 and SH2 domain.

Therefore, the structure of the SH3 domain in the domain pair is highly similar to the previously determined structure of the isolated domain showing that interdomain interactions have no significant effect on the structure of the SH3 domain itself. The dispersion of the chemical shifts of the SH2 domain in the domain pair is characteristic of an intact folded tertiary structure. The assigned ^{1}H, ^{13}C, ^{15}N chemical shifts of the SH3-SH2 domain pair of Lck have been deposited in the BioMagResBank, accession code 5794.

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