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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of lymphocyte specific kinase unique and SH3 domains

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Biological context

Human lymphocyte specific kinase (Lck) is a typical member of the Src-type tyrosine kinase family and consists of four functional domains, namely unique, SH3, SH2 and kinase. Whereas amino acid sequences of the other domains are highly conserved among different kinases, those of the unique domains are not. Moreover, 3D structures are known for SH3, SH2 and kinase domains of Lck (Eck et al., 1994; Hiroaki et al., 1996; Zhu et al., 1999) as well as for other tyrosine kinases. So far, however, no structure is known for the unique domain of any Src-type kinase. Lck unique domain is thought to serve as membrane anchor, but also plays a role in function and specificity of the other domains, e.g. SH2 and SH3 (Carrera et al., 1995). Because of its key role in T cell signaling and activation (for a review see Isakov and Biesinger, 2000), it is not surprising that pathogenic factors like human immunodeficiency virus (HIV) and herpes virus saimiri have evolved effector molecules that target Lck to ensure their own replication and persistence. In particular, HIV-1 Nef and herpes virus saimiri Tip directly bind to Lck SH3 domain. HIV-1 Nef also binds directly to CD4, which in turn binds directly to Lck unique domain. Thus, it is of utmost interest to study the 3D structure of Lck unique und SH3 domains as a whole to further investigate its interactions with pathogenic and host cell factors in the future. Therefore, we have undertaken a multidimensional heteronuclear NMR study of a recombinant peptide, consisting of the first 120 residues of human Lck. We report here the assignment of all but two backbone and most of the side-chain ^{1}H, ^{15}N and ^{13}C resonances of this peptide.

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Methods and experiments

A DNA fragment coding for the unique and the SH3 domain of Lck (residues 1 to 120, Lck-u3) was amplified by PCR and cloned into the vector pGEX-2T (Pharmacia Biotech) using *Bam*HI and *Eco*RI restriction sites introduced by PCR. Sequence analysis of the resulting expression plasmid pGEX-NT+SH3 confirmed 100% identity with human Lck (SwissProt KLCK_HUMAN, accession P06239; P07100). To avoid partial fragmentation due to two potential secondary cleavage sites for thrombin within Lck-u3 itself, the original expression system was not used for high level protein production. Instead, the *Bam*HI-*Eco*RI fragment was transferred to pGEX-6P-2 (Pharmacia Biotech) yielding the plasmid pLCKU3. This construct provides a PreScission protease cleavage site to remove the N-terminal glutathion-S-transferase (GST) affinity tag from the Lck-u3 peptide.

The GST-Lck-u3 fusion protein was expressed in *E. coli* strain BL21 transformed with pLCKU3. Cells with an O.D. at 600 nm of about 0.6 were induced with 1 mM IPTG and harvested after 3 h growth at 37 °C. For ^{15}N and/or ^{13}C labeling M9 minimal medium was used with ^{15}N-ammoniumchloride and ^{13}C glucose as sole nitrogen and carbon sources, respectively. Lck-u3 was purified using affinity chromatography on glutathione sepharose. On-column cleavage was performed using PreScission protease (Pharmacia). For

final purification, Lck-u3 was lyophilized, resolved in 2 ml 5 mM potassium phosphate buffer (pH 6.5) and applied to a Superdex 75 prepgrade gel filtration column on an FPLC system (Pharmacia), which was equilibrated with the same buffer.

NMR samples contained up to 1.6 mM protein in 20 mM sodium acetate, pH 6.5 containing 10 mM 2-mercaptoethanol with 10% D_2O. All NMR spectra were recorded at 298 K on Varian Unity INOVA spectrometers working at 600 or 750 MHz proton frequency equipped with Varian XYZ-PFG-$^1H\{^{13}C,^{15}N\}$ probes.

$^1H,^{15}N$-HSQC, HNCACB, CBCA(CO)NH, HNCO, HC(CO)NH-TOCSY, C(CO)NH-TOCSY and HCCH-COSY spectra were recorded to obtain resonance assignments. The accuracy of the Lck-u3 NMR assignments was further confirmed by sequential NOEs in the $^1H,^{15}N$-NOESY-HSQC spectrum. Figure 1 shows an example of strips taken from the $^1H,^{15}N$-NOESY-HSQC spectrum. Furthermore, $^1H,^{13}C$-HSQC and $^1H,^{13}C$-NOESY-HSQC spectra were acquired.

Preliminary inspection of chemical shift and NOE data yielded the presence of secondary structure elements known from other investigations of Lck SH3 in the absence of unique domain (Eck et al., 1994; Hiroaki et al., 1996). In addition, these data indicate the presence of helical secondary structure in the unique domain for residues 19 to 27 and 46 to 52.

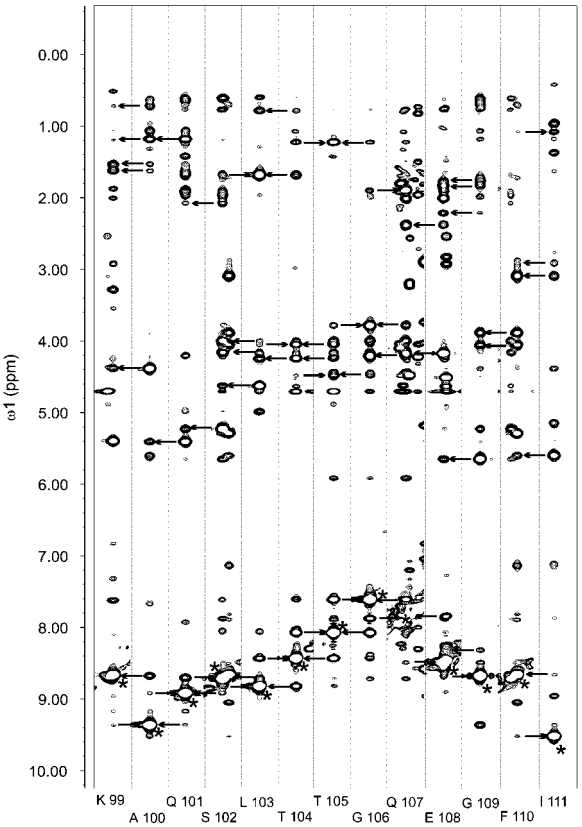

Figure 1. Composite of amide strips from K99 to I111 taken from the $^1H,^{15}N$-NOESY-HSQC spectrum (100 ms mixing time) of Lck-u3. Diagonal peaks are indicated by asterisks. Arrows pointing from intraresidual to interresidual cross resonances indicate sequential NOEs.

Extent of assignments and data deposition

The backbone ^1H, ^{15}N, ^{13}C and ^{13}CO assignments are essentially complete for Lck-u3. HN and ^{15}N resonances for 109 out of 111 (120 residues minus 9 prolines) possible amide resonances were assigned (98.2%) with the exception of His24 and Tyr25. Residues Tyr25 and Pro56 could not be assigned. Further, 98.3% (all except Tyr25 and Pro56) and 96.7% (all except Met1, Tyr25, Arg39 and Pro56) of $^{13}C\alpha$ and $^1H\alpha$ resonances could be assigned, respectively. The majority of side-chain resonances is assigned. The ^1H, ^{15}N and ^{13}C chemical shifts of the unique and SH3 domains of Lck-u3 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4860.

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