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Structure determination of human Lck unique and SH3 domains by nuclear magnetic resonance spectroscopy

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Abstract

Background: Protein tyrosine kinases are involved in signal transduction pathways that regulate cell growth, differentiation, activation and transformation. Human lymphocyte specific kinase (Lck) is a 56 kDa protein involved in T-cell- and IL2-receptor signaling. Three-dimensional structures are known for SH3, SH2 and kinase domains of Lck as well as for other tyrosine kinases. No structure is known for the unique domain of any Src-type tyrosine kinase.

Results: Lck(1–120) comprising unique and SH3 domains was structurally investigated by nuclear magnetic resonance spectroscopy. We found the unique domain, in contrast to the SH3 part, to have basically no defined structural elements. The solution structure of the SH3 part could be determined with very high precision. It does not show significant differences to Lck SH3 in the absence of the unique domain. Minor differences were observed to the X-ray structure of Lck SH3.

Conclusion: The unique domain of Lck does not contain any defined structure elements in the absence of ligands and membranes. Presence of the unique domain is not relevant to the three-dimensional structure of the Lck SH3 domain.

Background

Protein tyrosine kinases are involved in signal transduction pathways that regulate cell growth, differentiation, activation and transformation. Human lymphocyte specific kinase (Lck) is a 56 kDa protein involved in T-cell- and IL2-receptor signaling. 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, three-dimensional structures are known for SH3, SH2 and kinase domains of Lck [1–4] 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 a membrane anchor, but also plays a role in function and specificity of the other domains, e.g. SH2 and SH3 [5]. Further, Lck unique domain binds to the cytoplasmic regions of CD4 and CD8α via cysteines and a divalent cation [6,7]. Because of its key role in T cell signaling and activation (for a review see [8]), it is not surprising that pathogenic factors like human immunodeficiency virus (HIV) and *Herpesvirus saimiri* have evolved effector molecules that target Lck to ensure their own replication and persistence. In particular, HIV-1 Nef and *Herpesvirus saimiri* Tip proteins 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 interest to study the three-dimensional structure of Lck unique und SH3

domains as a whole, which is done in the present study by nuclear magnetic resonance (NMR) spectroscopy.

Results

Earlier we reported almost complete assignments for ^{1}H, ^{13}C, and ^{15}N resonances of human Lck unique and SH3 domains [9]. During the NOE-assignment process it became obvious that unique and SH3 domains would differ remarkably in their content of secondary structural elements and tertiary structure. While a large number of medium- and long-range NOEs could be identified in the SH3 part of the molecule (Fig. 1A), only intraresidual or sequential NOEs were found in the unique domain. To clarify, whether the unique part contains stable structural elements at all, we determined heteronuclear amide NOE values (Fig. 1B) for most of the residues in Lck(1–120). In contrast to the unique domain, most residues of the SH3 domain show heteronuclear amide NOE values larger than 0.6.

For the SH3 part, a total of 1817 NOE distance constraints, including 610 long-range NOEs (Table 1), were derived from three-dimensional ^{15}N or ^{13}C edited NOESY spectra recorded from uniformly ^{15}N and ^{13}C isotope labeled recombinantly expressed Lck(1–120) protein. These experimental restraints were taken as input for simulated annealing and refinement calculations. In addition, 23 residues showed $^{3}J_{HNH\alpha}$ coupling constants either smaller than 6.0 Hz or larger than 8.0 Hz and the respective backbone torsion angles were therefore restrained to adopt values between -80° to -40° or between -160° and -80°, respectively. Together, 25 structures were obtained that did not show any NOE distance violation greater than 0.2 Å. The root mean squared deviation of these 25 structures relative to their average structure was 0.16 Å and 0.67 Å for backbone and all heavy atoms, respectively. That means, the resulting structure is rather well defined as seen in the overlay of all 25 protein backbone and side chain atoms (Fig. 2A). Because only residues of the SH3 part of the molecules contributed to experimental data used for the structure calculations, the unique part is not displayed on the figures describing the structure of Lck(1–120).

Large average local displacement values relative to the mean structure indicate either local flexibility of the protein or lack of experimental data for this region. Evaluation of these values for Lck SH3 solution structure indicate regions Leu69-Gly81, Gln93-Glu96, and Asn114Val116 of the protein to be less defined (Fig. 1C). The first two regions have also slightly decreased heteronuclear NOE values (Fig. 1B) indicating increased dynamic behavior of the residues therein. Those regions also show minor differences between the solution structure and the crystal structure (Fig. 2C). We deposited our data in the PDB under accession code 1KIK.

Discussion

^{1}H-^{15}N-heteronuclear amide NOE values are a measure for the dynamics of the local environment within the time scale of the absolute nuclear magnetic resonance (NMR) frequencies (60 to 750 MHz in the present study). While negative ^{1}H,^{15}N-Hetero NOE values indicate flexible, unstructured regions, stabilizing structural elements give rise to values above 0.6 [10]. As can be seen from figure 1B, residues of the unique domain show increased dynamic behavior. In addition, while a large number of medium- and long-range NOEs could be identified in the SH3 part of the molecule, only intraresidual or sequential NOEs were found for protons in the unique domain (fig. 1A). Our data clearly show that the complete unique domain is absent of any stable structural element. From our data, however, this can only be inferred for the absence of membranes or ligands. Whether the unique domain adopts a defined tertiary structure in the presence of Lck SH2 or kinase domains, a ligand, or when anchored in the membrane, remains to be determined.

In contrast to the unique domain, most residues of the SH3 domain show ^{1}H,^{15}N-heteronuclear amide NOE values larger than 0.6, indicating that this part of the molecule contains stable structural elements. Thus, only the SH3 part of the molecule was object to further evaluation of structural data. Together, 25 structures were obtained from simulated annealing molecular dynamics calculations based on experimental conformational restraint data. The obtained structures did not show any NOE distance violation greater than 0.2 Å. The root mean square deviation of these 25 structures relative to their average structure was 0.16 Å and 0.67 Å for backbone and all heavy atoms, respectively. That means, the resulting structure is rather well defined as seen in the overlay of all 25 protein backbone and side chain atoms (Fig. 2A).

The solution structure of Lck SH3 is very similar to the crystal structure of Lck SH3 [1] (Fig. 2C). It exhibits a typical SH3 fold consisting of five antiparallel β-strands (βA: Leu65 to Ala68; βB: Gln87 to Glu92; βC: Trp92 to Ser102; βD: Glu108 to Pro112 and βE: Val116 to Lys118, Fig. 2B), that are forming two β-sheets packed at almost right angles, with strand βB participating in both sheets. A 3_{10}-helix formed by residues Phe113 to Phe115 was detected in two of 25 structures. This may suggest that the 3_{10}-helix frequently observed in crystal structures of SH3 domains is not necessarily present in all of the molecules in solution. It may, however, indicate as well that this is only due to lack of experimental data for this region (fig. 1A), leading to increased local displacement values (fig. 1B) despite high heteronuclear NOE values (fig. 1C) for residues

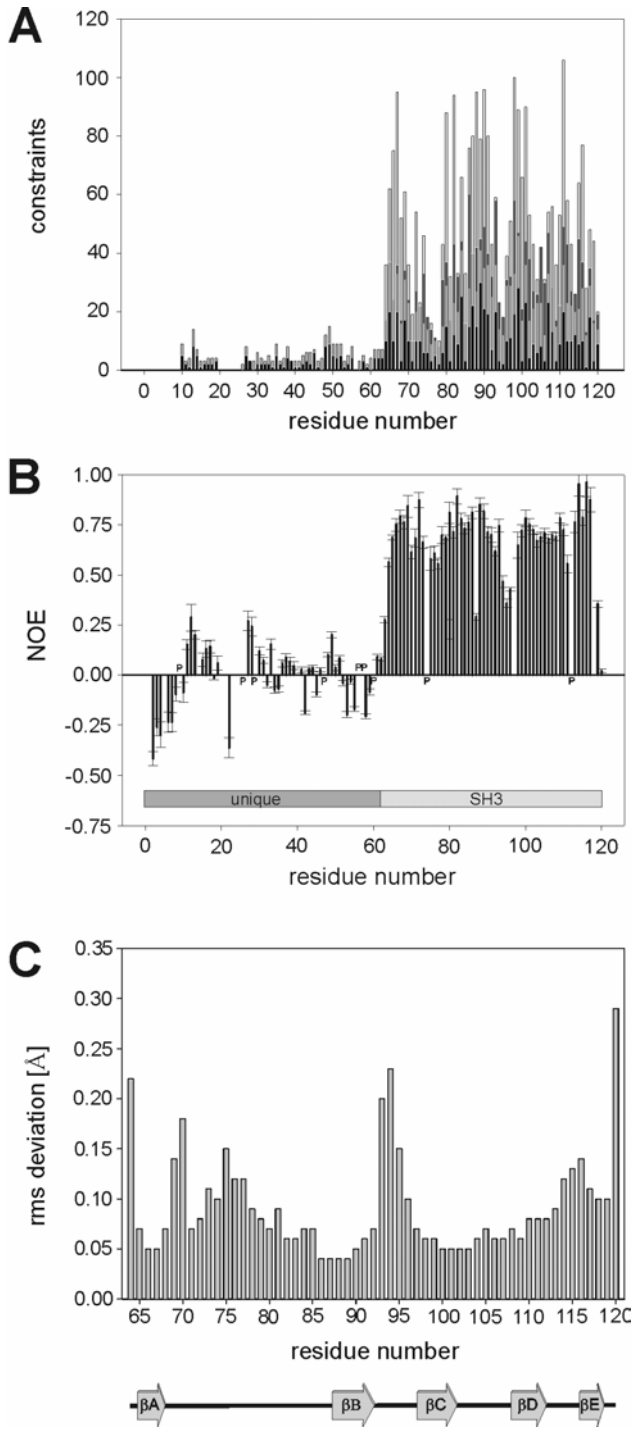

Figure 1

Number of experimental distance constraints per residue and dynamic behavior of Lck unique and SH3 domains, as well as local precision of SH3 domain conformation. A: Number of intraresidual (black), sequential (light gray), medium (dark gray) and long range (white) NOE distance constraints per residue of Lck(1–120). **B**: Heteronuclear ^{1}H-^{15}N-NOE values of Lck(1120) amide resonances. Proline residues are indicated (P), the position of the unique and the SH3 domains are marked with labeled horizontal bars. **C**: Average local displacement values among the 25 obtained solution structures of Lck(64–120). For each three-residue window the average displacement of the backbone atoms was calculated and plotted against the residue number that corresponds to the central residue of the window. Location of secondary structure elements is given at the bottom.

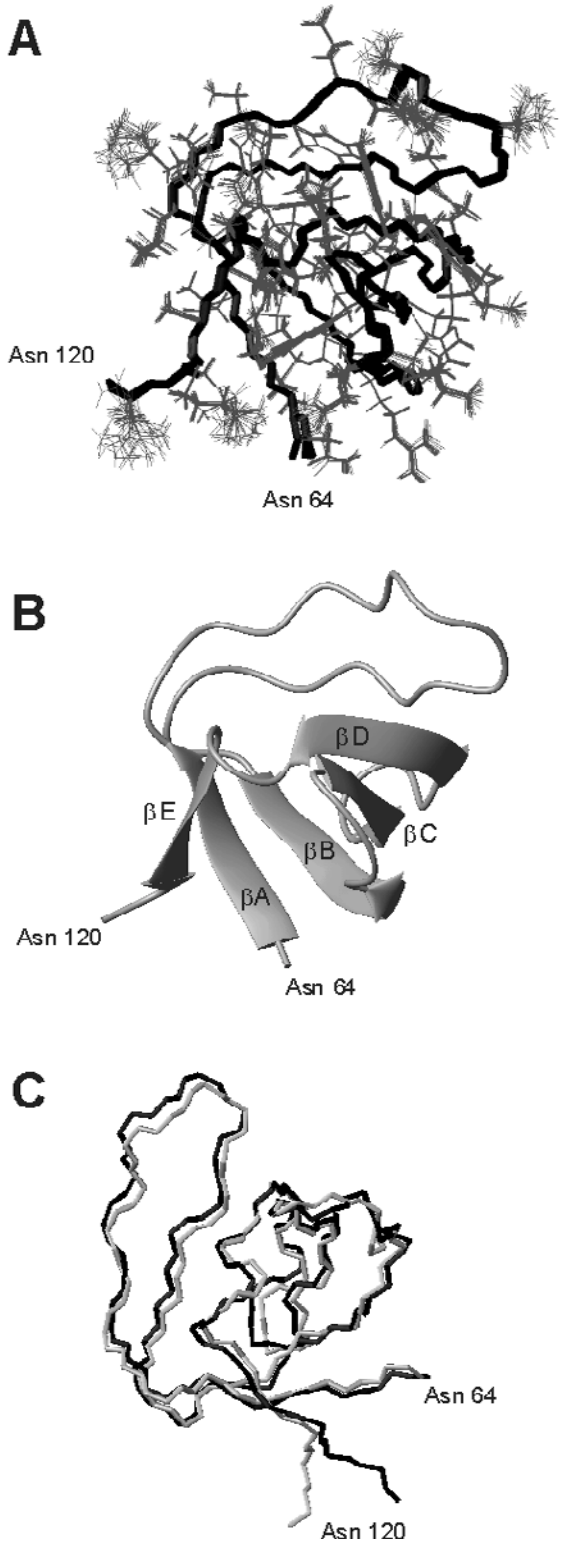

Figure 2
Representation of Lck(64–120) structure. A: Overlay of 25 structures of Lck(64–120). Structures were fitted to backbone atoms of residues 64 to 120. The backbone is colored in black, side chains are colored in light gray. **B**: The structure of Lck(64–120) is shown as ribbon diagram. **C**: Superposition of the crystal structure (black) [1] and the solution structure (gray) with the lowest energy of the Lck SH3 domain.

Table 1: Constraints and structural statistics for the resulting 25 NMR structures of Lck(1–120)

Number of experimental restraints:	
total number of assigned NOEs	1817
intraresidual (|i-j| = 0)	635
interresidue sequential (|i-j| = 1)	357
interresidue medium range (1 < |i-j| 5)	215
long range (|i-j| > 5)	610
X-PLOR energies (kcal/mol)	
total	234.7 ± 1.13
bond	17.34 ± 0.27
angle	88.15 ± 0.79
impropers	10.91 ± 0.53
Van-der-Waals	56.01 ± 0.92
NOE	62.26 ± 0.88
RMS deviations to the mean structure (Å)	
backbone heavy atoms	0.16 ± 0.04
all heavy atoms	0.67 ± 0.09
RMS deviations to experimental constraints and idealized geometry	
NOE (Å)	0.0212 ± 0.0001
bond (Å)	0.0031 ± 0
angle (Å)	0.4183 ± 0.0019
impropers (°)	0.2721 ± 0.0066
Φ, Ψ angles consistent with Ramachandran plot (%)	
most favored regions	70.9
allowed regions	100
generously allowed regions	100

around Phe115. Another NMR study on Lck SH3 that was published during the time course of the present study, also did not detect such a 3_{10}-helix [4].

The root mean square deviation (rmsd) value for the backbone coordinates of residues Asn64 to Asn120 is 1.05 Å between the hereby reported solution and the crystal [1] structure. The coordinates for the very carboxyl terminal residues, however, differ remarkably. Comparison with a previously reported solution structure of Lck SH3 domain [2] was not possible due to the lack of data deposition in the Brookhaven Protein Data Bank (PDB).

Conclusions

Employing recombinant human Lck comprising its unique and SH3 domains, we determined the solution structure of Lck SH3 with very high precision in the presence of Lck unique domain. We report for the first time structural aspects of a Src-type tyrosine kinase unique domain. In the absence of membranes or ligands, Lck unique domain does not contain any stable structural elements. Whether Lck unique domain adopts any defined structure in the presence of membranes or interaction partners like CD4 or CD8α, remains to be clarified by future investigations.

Methods

Protein purification

Expression and purification of residues 1 to 120 of human Lck comprising its unique and SH3 domains (LckU3) was carried out exactly as described earlier [9,11]. Identity of the proteins was confirmed by MALDI-TOF-MS and N-terminal amino acid sequence analysis.

NMR spectroscopy

NMR samples contained 1.6 mM protein in 20 mM sodium acetate, 150 mM NaCl, pH 6.5, in 90 % H_2O/10 % D_2O with 10 mM 2-mercaptoethanol and 0.02 % (by weight) sodium azide. NMR spectra were recorded at 298 K on Varian Unity INOVA spectrometers equipped with a triple-axis pulse-field-gradient (PFG) $^{1}H/^{15}N/^{13}C$ probe at proton frequencies of 600 and 750 MHz. The resonance assignment of LckU3 was previously described [9]. Structural constraints were derived from ^{15}N-edited NOESY-

HSQC (100-ms mixing time) [12] and aliphatic ^{13}C-edited NOESY-HSQC (80-ms mixing time) experiments [13] in the described buffer. Uniformly ^{13}C-^{15}N-labelled protein was used for these experiments. ^{15}N-labelled protein was employed for the ^{1}H-^{15}N-heteronuclear NOE experiments [14].

Data evaluation and structure calculation

Based on the almost complete assignment of ^{1}H, ^{13}C, and ^{15}N resonances of LckU3, a total of 1817 NOE distance constraints (including 610 long-range NOEs) could be derived from three-dimensional NOESY spectra in an iterative procedure (Table 1). NOE analysis and assignment was performed using XEASY [15] and ARIA [16]. Interproton distances were used directly to calibrate experimental peaks and to extract distance constraints [16]. Lower and upper bounds for distance constraints were derived from the target distances empirically by estimation of the error as 12.5% of the target distance squared. Distances involving ambiguous constraints, methyl groups, aromatic ring protons and the non-stereospecifically assigned methylene protons were treated as sum of separate contributions to the target function, known as "sum averaging" [17]. Final structures were calculated using the simulated annealing protocol with CNS version 1.0 [18] using standard parameters with the following modifications: For conformational space sampling 20 ps with a time step of 10 fs were simulated using torsion angle dynamics at a temperature of 50000 K, followed by 30 ps of slow cooling to 0 K with a time step of 15 fs. In an additional Cartesian slow cooling stage, the temperature was decreased for 20 ps from 2000 K to 0 K with a time step of 5 fs. After simulated annealing the structures were subjected to 2000 steps of energy minimization. A total of 25 structures that did not show any distance constraint violation of more than 0.2 Å was used for further analysis. Geometry of the structures, structural parameters and secondary structure elements were analyzed and visualized using the programs MOLMOL [19] and PROCHECK [20]. The coordinates of the SH3 domain have been deposited in the Protein Data Bank, with accession code 1KIK.

List of abbreviations

Lck: lymphocyte specific kinase.

NMR: nuclear magnetic resonance.

Src: Src kinase.

SH2: Src homology domain 2.

SH3: Src homology domain 3.

NOE: nuclear Overhauser enhancement.

NOESY: NOE spectroscopy.

HSQC: heteronuclear single quantum coherence.

LckU3: Lck part comprising unique and SH3 domains.

MALDI-TOF-MS: Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry.

Authors' contributions

LB prepared the isotope labeled protein, carried out the NMR experiments and did all the spectra evaluation and structure calculations. DW planned the design of the study, drafted the manuscript, coordinated the experiments, and did part of the data evaluation. Both authors read and approved the final manuscript.

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