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# Letter to the Editor: $^{1}$H, $^{13}$C and $^{15}$N chemical shift assignments of the SH2 domain of the Csk homologous kinase

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## Biological context

The Csk homologous kinase (CHK), also known as Ctk/Hyl/Matk/Lsk, is an intracellular protein tyrosine kinase that is highly expressed in the haematopoietic system and the brain. Along with its close relative Csk (C-terminal Src kinase), the *in vivo* role of CHK is to specifically phosphorylate the C-terminal tails of Src family of protein tyrosine kinases, resulting in the stabilisation of their inactivate form. Tight and exquisitely tuned control of Src family activity is fundamental to many processes and loss of control of Src family activity is often associated with cancer (reviewed in Lowell and Soriano, 1996). Although the molecular mechanism of kinase regulation in Csk and CHK themselves is unclear, the recent crystal structure of intact Csk reveals that the linker regions on either side of the Src homology-2 (SH2) domain make intimate contact with the N-terminal lobe of the kinase domain and may play a direct roles switching between active and inactive forms of the enzyme (Ogawa et al., 2002).

The SH2 domain in CHK has been implicated in targeting the enzyme to specific phosphotyrosine containing sequences in the cytoplasmic regions of several cell surface receptors, including c-KIT, ErbB-2/neu, and TrkA (Price et al., 1997; Zrihan-Licht et al., 1998; Yamashita et al., 1999). The increased expression of ErbB-2/neu, with a concomitant increase in Src kinase activity, is correlated with the onset of breast cancer. It has been demonstrated that CHK, but not Csk, is capable of inhibiting the transformation and invasiveness of breast cancer cells by down-regulating ErbB-2/neu-activated Src kinases (Bougeret et al., 2001). The level of Src kinase inhibition is positively correlated with the strength of the SH2 affinity for ErbB-2/neu (Kim et al., 2002), although it is unclear if this is due to a direct effect on the kinase activity of CHK or more efficient targeting of CHK to the receptor.

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## Methods and experiments

To further investigate the role of the SH2 and its flanking linker regions we have generated a 123-residue construct composed of residues 69–186 of rat CHK (NCBI I56579) with the non-native sequence GPLGS (arising from the pGEX-6P-3 vector (Phamacia)) at its N-terminus. The *E. coli* expressed glutathione S-transferase fusion protein was purified by glutathione affinity chromatography. The fusion protein was then proteolytically cleaved and the CHK SH2 was purified by sequential Mono Q anion-exchange and Mono S cation-exchange chromatography. The correct identity and extent of isotope incorporation (>99%) in the final products was confirmed by MALDI-TOF mass spectrometry. Unlabelled, $^{15}$N-labelled and $^{13}$C,$^{15}$N-labelled samples were prepared in 10 mM phosphate buffer pH 7.0, 100 mM NaCl, 1–2 mM dithiothreitol, 0.02% $NaN_3$ with the addition of $^{2}H_2O$ to 10% v/v. Final protein concentrations ranged from 0.2–0.5 mM and samples were stored under nitrogen in flame-sealed 5 mm NMR tubes.

NMR experiments were recorded at 25 °C on Varian Inova 500 and 600 MHz spectrometers. Backbone and side chain assignments were made using a combination of homonuclear 2D-NOESY, TOCSY and DQF-COSY data and 3D-TOCSY-HSQC, NOESY-HSQC, HNHA, HNHB, HNCO, HN(CA)CO, CBCANH, CBCA(CO)NH, HCCH-TOCSY and HC(CO)NH data.

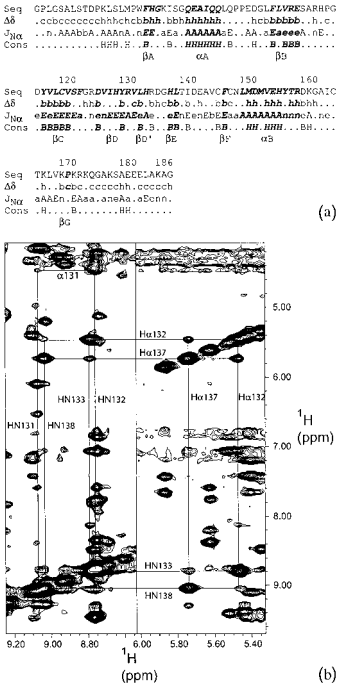


*Figure 1.* (a) Position of secondary structure elements in the CHK SH2. Seq: Rat amino acid sequence with bold italics showing the corresponding position of secondary structure elements from the human x-ray structure (PDB 1JWO). Δδ: Consensus of secondary structure prediction from $^{1}$Hα, $^{13}$Cα, $^{13}$Cβ, $^{13}$CO chemical shift values according to the ranges described in Wishart et al. (1992) and Wishart and Sykes (1994). The symbols c (coil), b (beta-strand) and h (helix) indicate when at least three of four (for non-Gly), or two of three (for Gly) chemical shift deviations are in agreement. A dot indicates no consensus. $J_{N\alpha}$: The value of the $^{3}J_{HN-H\alpha}$ coupling constant is indicated by the symbols; A: $J \leq 5$ Hz; a: 5 Hz $< J \leq 6$ Hz; n: 6 Hz $< J \leq 7$ Hz; e: 7 Hz $< J < 8$ Hz; E: $J > 8$ Hz. A dot indicates an unmeasured value. Cons: The consensus between Δδ and $J_{N\alpha}$ is indicated by H (helix): When h is aligned with a or A; and B (beta): When b is aligned with e or E. All other combinations are represented by a dot. (b) Regions of the homonuclear 2D-NOESY spectrum of CHK SH2. Resonances arising from Leu$^{131}$, His$^{132}$, Arg$^{133}$, Leu$^{137}$ and Thr$^{138}$ are joined by lines. The presence of Hα-Hα NOES between His$^{132}$ and Leu$^{137}$, HN-Hα NOEs between Arg$^{133}$ and Leu$^{137}$ and Hα-HN NOEs between Arg$^{132}$ and Leu$^{138}$ and are diagnostic of hydrogen bonding between Leu$^{131}$ and Thr$^{138}$ in an anti-parallel arrangement.

## Extent of assignments and data deposition

We have established complete backbone $^{1}$H, $^{15}$N, $^{13}$Cα and $^{13}$CO assignments for all residues with the exception of the N-terminal Gly (no assignments) and Pro$^{97}$ (no $^{13}$C assignments) in the Pro$^{97}$-Pro$^{98}$ dyad. For the side chains, all non-exchangeable side chain hydrogens were assigned along with all $^{13}$Cβ shifts with the single exception of Pro$^{97}$. $^{1}$H and $^{15}$N assignments were obtained for the indole group of Trp$^{81}$ and all Asn and Gln side chain amides, but no Arg guanidinium groups could be unambiguously assigned. A single hydroxyl proton, that of Thr$^{156}$ was in slow exchange and able to be assigned. There is evidence for peptide bond *cis-trans* isomerization involving Pro$^{97}$-Pro$^{98}$ at the apex of the AB-loop. The chemical shift and $^{3}J_{HN-H\alpha}$ coupling data have been deposited in the BioMagResBank accession number 5504.

Figure 1a shows the pattern of secondary structure elements, as revealed by analysis of $^{3}J_{HN-H\alpha}$ values and the deviation of Cα, Cβ, CO and Hα chemical shifts from random coil values (Wishart et al., 1992; Wishart and Sykes, 1994). In general, the location of the secondary structure elements is consistent with the canonical SH2 fold determined for the human sequence (PDB 1JWO unpublished; residues 117–213 of human CHK, 95% identity with rat over the corresponding region). However, analysis of inter-strand Hα-Hα and Hα-HN NOE patterns indicate that the register of β-strand E in 1JWO is incorrect. The NOE data (Figure 1b) are consistent with antiparallel inter-strand hydrogen bonds between residues Leu$^{131}$ in strand D′ and Thr$^{138}$ in strand E (corresponding to residues Leu$^{172}$ and Thr$^{179}$ in 1JWO, rather than Leu$^{172}$ and Leu$^{178}$) and the presence of hydrogen bonding between Leu$^{137}$ in strand E and Phe$^{145}$ in strand F (corresponding to Leu$^{178}$ and Phe$^{186}$ in 1JWO, rather than His$^{177}$ and Phe$^{186}$). Our analysis indicates that there is one extra residue in the DE-loop and one less residue in the EF-loop, explaining the presence of bad steric contacts and grossly distorted bond angles in these regions of the publicly available human CHK SH2 crystal structure.

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