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ARTICLE

Backbone NMR resonance assignment of the Abelson kinase domain in complex with imatinib

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Abstract Imatinib (Glivec or Gleevec) potently inhibits the tyrosine kinase activity of BCR-ABL, a constitutively activated kinase, which causes chronic myelogenous leukemia (CML). Here we report the first almost complete backbone assignment of c-ABL kinase domain in complex with imatinib.

Keywords Tyrosine kinases · Glivec · BCR-ABL · Chronic myelogenous leukemia · Selective labeling

Biological context

Tyrosine kinases are important mediators in signal transduction pathways and are tightly regulated by several mechanisms. Aberrant activation of these enzymes may lead to diseases, and mutations in kinase genes are implicated in many forms of cancer (Greenman et al. 2007). A number of tyrosine kinases are therefore attractive drug targets for the discovery of inhibitors, which can modulate the activity of these enzymes. The BCR-ABL fusion protein, having a constitutively activated Abelson (ABL) kinase domain, is one such important drug target, which has been clinically validated by imatinib (Glivec; Novartis Pharma AG), an efficacious and well-tolerated treatment for chronic myelogenous leukemia (CML) (Ren 2005). Much structural knowledge on ABL-inhibitor complexes has been generated using X-ray crystallography (Nagar et al. 2002; Cowan-Jacob et al. 2007), but very little is known about the solution structure and dynamics of ABL kinase.

To enable NMR studies on the solution behavior of ABL kinase, we have obtained backbone resonance assignment of ABL kinase domain in complex with imatinib. The selected construct of the ABL kinase domain (denoted here as ABL, residues GAMDP-S229-S500) contains 277 residues and has a typical kinase bilobal structure, with the N-terminal lobe containing β-sheets and the conserved helix C, and the C-terminal lobe being mainly helical. At the interface of the two lobes is the ATP-binding pocket, which is also the binding site of most ABL inhibitors.

Methods and materials

Since ABL is not readily expressed in *E. coli*, we have developed a protocol for the $^{13}C/^{15}N$ isotope labeling of the ABL kinase catalytic domain in Baculovirus-infected insect cells, either uniformly or selectively for certain amino acid types (Strauss et al. 2003; Strauss et al. 2005).

Uniformly $^{13}C,^{15}N$ or selectively labeled samples (Strauss et al. 2003; Strauss et al. 2005) of the ABL-imatinib complex were prepared as 0.4 mM solutions in 250 µl (Shigemi microtubes) of buffer containing 95% H_2O, 5% D_2O, 20 mM BisTris, 100 mM NaCl, 2 mM EDTA, 3 mM DTT at pH 6.5 with an ABL:imatinib ratio of 1:1. NMR spectra were recorded at 293 K on Bruker

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DRX 600 (cryo or room temperature probe) or DRX 800 MHz (cryoprobe) spectrometers. Due to the lack of deuteration, the sensitivity of NMR experiments involving $^{13}C^{\beta}$ or other side chain nuclei was very low. Therefore, backbone assignments had to be performed with non-TROSY versions of the HNCO, HNCA, HN(CO)CA (Grzesiek and Bax 1992), and an ^{15}N-edited ^{1}H-^{1}H NOESY. The assignment was aided and verified by spectra of a total of 15 selectively labeled samples that had ^{15}N, $^{13}C^{\alpha}$ and $^{13}C'$ nuclei introduced specifically for certain amino acid types (see supplementary material Table 1). All NMR data were processed and analysed using the NMRPipe (Delaglio et al. 1995) and NMRView (Johnson and Blevins 1994) software suites. ^{1}H, ^{15}N and ^{13}C are referenced relative to the frequency of the ^{2}H lock resonance of water.

Extent of assignment and data deposition

The achieved assignments comprise 96% of all backbone $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$ and ^{13}CO resonances, covering 254 of the 264 non-proline residues (Fig. 1). Unassigned residues consist of the *N*-terminal glycine, seven residues within the activation loop (consisting of residues D381-P402), and H361. Line broadening of adjacent residues indicates that most of the missing residues are broadened beyond detection due to intermediate conformational exchange. Despite the difficulty in observing the entire activation loop, the assignments include at least 14 key residues involved in ligand binding. The assignments have been deposited in the BMRB (accession number 15488).

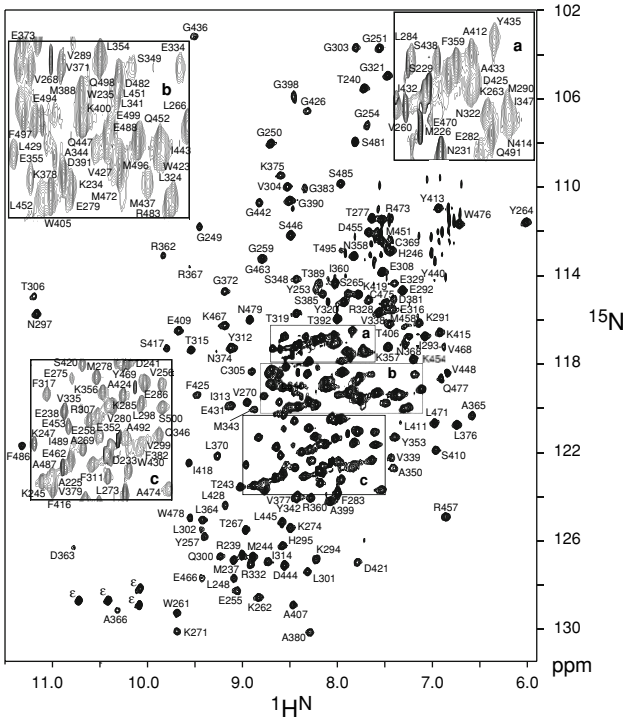

Fig. 1 $^{1}H^{N}$-^{15}N HSQC-TROSY (Pervushin et al. 1997) spectrum of uniformly ^{15}N-labeled ABL kinase domain-imatinib complex with assignment information. An 'ε' denotes unassigned W^{ε} NH sidechain resonances, Subpanels a, b and c show enlarged regions of the respective rectangular boxes in the main spectrum

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