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ARTICLE

# $^{1}H$, $^{13}C$, $^{15}N$ backbone and side-chain resonance assignments of the human Raf-1 kinase inhibitor protein

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**Abstract** Raf-1 kinase inhibitor protein (RKIP) plays a pivotal role in modulating multiple signaling networks. Here we report backbone and side chain resonance assignments of uniformly $^{15}N$, $^{13}C$ labeled human RKIP.

**Keywords** NMR resonance assignments · RKIP · Secondary structure prediction

## Biological context

Raf-1 kinase inhibitor protein (RKIP, 187aa), initially identified as a phosphatidylethanolamine binding protein (PEBP) based on its binding property, is a member of the phosphatidylethanolamine-binding protein (PEBP) family (Schoentgen et al. 1992) which is a highly conserved group of proteins with homologues in a wide variety of organisms,from bacteria to plants and mammals. RKIP is widely expressed in most tissues at various developmental stages, including brain, testis, epididymis, liver and kidney (Frayne et al. 1998; Frayne et al. 1999).

In recent years, there has been an increased interest in RKIP due to its pivotal role in modulating multiple signaling networks (Trakul and Rosner 2005). It has been well established that RKIP regulates mitogen activated protein kinase (MAPK) pathways, which is essential for cellular proliferation, differentiation, apoptosis, survival and migration (Pearson et al. 2001; Wellbrock et al. 2004). Moreover, RKIP deregulation would lead to many human diseases such as cancer, and developmental disorders (Dhillon et al. 2007; Roberts and Der 2007). Interestingly, in its non-phosphorylated form, RKIP binds with Raf-1 (a member of the MAP kinase kinase kinase family) and inhibits Raf-1-mediated phosphorylation of MEK, and then attenuate the signaling downstream of MEK. However, once RKIP is phosphorylated by protein kinase C (PKC) at the phosphorylation site (serine 153) (Corbit et al. 2003; Lorenz et al. 2003), it dissociates from Raf-1 and turns to bind and suppress G-protein-coupled receptor kinase 2 (GRK-2), a negative regulator of G-protein coupled receptors (GPCRs) (Kroslak et al. 2001). In its active state, GRK-2 phosphorylates GPCRs, uncoupling them from their associated G-proteins and marking them for degradation. By blocking the activity of GRK2, RKIP stimulates signaling through GPCRs and influences processes such as cardiac physiology. Therefore, the phosphorylation-dependent activity of RKIP is associated with two biologically important cellular signaling systems.

Although it has been well known that RKIP can bind with either Raf-1 or GRK2, the underlying molecular mechanisms of these intermolecular interactions remain to be addressed. Here we report backbone and side-chain resonance assignments as well as the secondary structure prediction for the human RKIP protein. Our work provides the basis for the detailed structural investigation of the interactions between RKIP and either Raf-1 or GRK2.

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## Methods and experiment

### Expression and purification

The expression vector harboring the human RKIP (hRKIP) gene, pReceiver-B01a-hRKIP, was transformed into *E. coli* BL21(DE3). The sequence was preceded by a histidine tag (residues (MSYYHHHHHHEGVRT) for purification purposes. Uniformly $^{15}$N, $^{13}$C-labelled samples were produced by growing cells at 25°C in M9 minimal medium, contains $^{15}NH_4Cl$ and $^{13}C_6$-glucose as sole sources of nitrogen and carbon, respectively. The protein was induced with 0.4 mM isopropyl $\beta$-D-thiogalactopyranoside and expressed for 16 h. The culture was harvested by centrifugation (11,000 rpm, 3 min, 4°C), and resuspended in buffer A (50 mM $NaH_2PO_4$, 300 mM NaCl, pH 8.0, and 1 mM PMSF), then the suspension was lysed by sonication and centrifugation (11,000 rpm, 30 min, 4°C). The supernatant was loaded onto a Ni–NTA affinity column (Qiagen). After washing the captured column sequentially with 20 mM, 60 mM imidazole, the His-tag hRKIP protein was eluted with 250 mM imidazole. The purity of hRKIP was checked to be 95% by SDS–PAGE (15% gel).

### NMR spectroscopy

Uniformly $^{15}$N-and $^{13}$C/$^{15}$N-labelled samples of hRKIP were exchanged to NMR buffer and concentrated to about 3 mM in NMR buffer (200 mM sodium acetate, 5 mM DTT, 10% $D_2O$ (v/v) and 0.1 mM sodium azide, pH 4.0). A suit of 3D heteronuclear NMR spectra were recorded at 25°C on a Varian Unity Inova 600 MHz spectrometer equipped with a triple resonance, z-axis gradient probe, including HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, HBHA(CO)NH, H(CCO)NH, C(CO)NH, HCCH-TOCSY, CCH-TOCSY, $^{15}$N-edit NOESY-HSQC, $^{13}$C-edit NOESY-HSQC, 15N-edit TOCSY-HSQC. NMR spectra were processed using the program NMRPipe (Delaglio et al. 1995) and analyzed with the program SPARKY (Goddard and Kneller, San Francisco, CA).

### Extent of assignments and data deposition

A 2D 1H-15N HSQC spectrum of hRKIP is well-dispersed, suggesting that the protein is well structured (Fig. 1). Ninety-five percent of all backbone $^{1}$H, $^{13}$C, $^{15}$N resonances were assigned for all non-proline residues except

**Fig. 1** Assigned 15N-1H HSQC spectrum of the human RKIP protein recorded at 600 MHz 1H frequency and 25°C

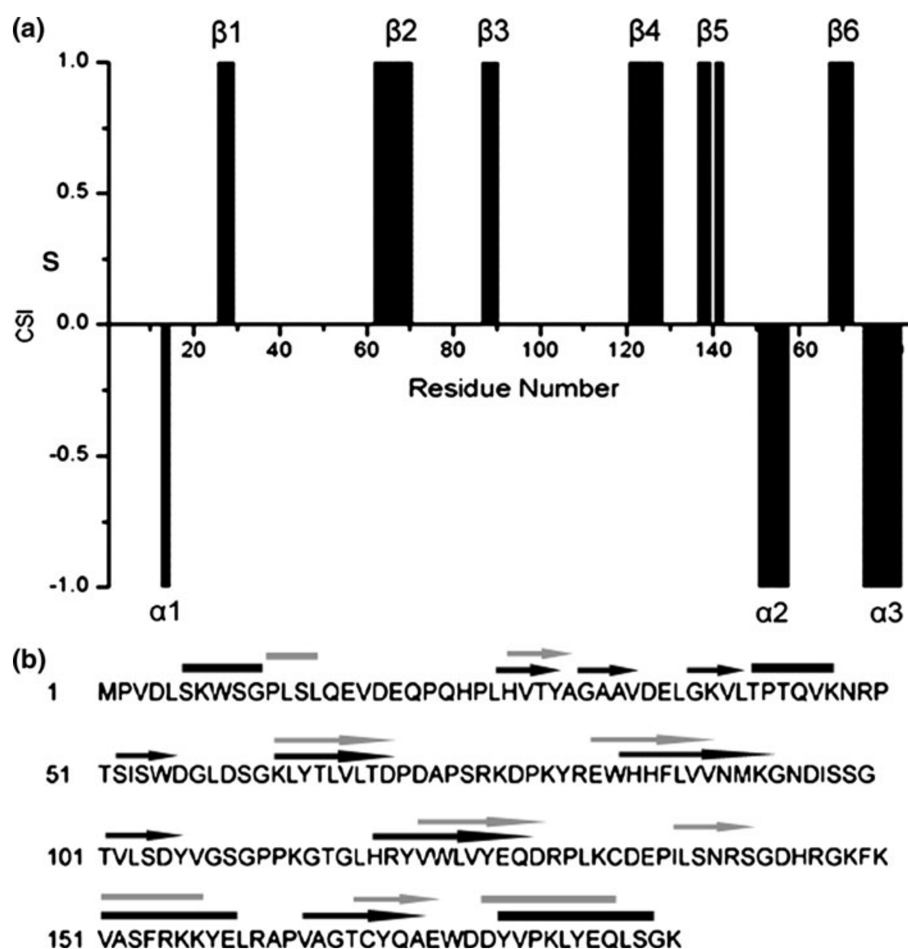


**Fig. 2** Secondary structure of the human RKIP protein. **a** The consensus chemical shift index ($H_{\alpha}$, $C_{\alpha}$, $C_{\beta}$ and C′) for the human RKIP protein. Values of +1, 0 and −1 indicate $\beta$-sheet, random coil and $\alpha$-helical structure, respectively. **b** Secondary structure for the human RKIP protein as obtained from the crystal structure (*black arrows and rectangles*) and from the CSI approach (*grey arrows and rectangles*). *Arrow*s represent the $\beta$-strands and rectangles the helices

the N-terminal His-tag. Eighty percent of side-chain resonance assignments were completed, including 90% $^{13}C$ and 85% 1H chemical shifts with the exception of aromatic side chain. Chemical shifts were deposited in the BioMagResBank under the access number BMRB 16992.

The secondary structure of hRKIP was predicted to contain six $\beta$-strands (residues 26–29, 62–70, 83–90, 121–128, 137–142, 167–172) and three helix (residues 11–14, 151–157, 175–183) using the chemical shift index (CSI) approach (Wishart and Sykes 1994) based on the obtained $H_{\alpha}$, $C_{\alpha}$, $C_{\beta}$, and C′ chemical shifts (Fig. 2a), overall in agreement with the crystal structure of the human RKIP protein (Banfield et al. 1998) (Fig. 2b).

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