Journal of Biomolecular NMR, **26:** 373–374, 2003.
KLUWER/ESCOM

Letter to the Editor: Rapid backbone ^{1}H, ^{13}C, and ^{15}N assignment of the V1 domain of human PKC iota using the new program IBIS

Michael H.A. Roehrl[a,c], Sven G. Hyberts[a], Zhen-Yu Jim Sun[a], Alan P. Fields[b] & Gerhard Wagner[a,*]

[a]*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, U.S.A.;* [b]*Sealy Center for Oncology and Hematology, University of Texas Medical Branch, Galveston, TX 77555, U.S.A.;* [c]*Graduate Program in the Biological and Biomedical Sciences, Division of Medical Sciences, Faculty of Arts and Sciences, Harvard University, 220 Longwood Avenue, Boston, MA 02115, U.S.A.*

Received 7 January 2003; Accepted 11 March 2003

Biological context

Leukemia is a malignant disease of the bone marrow that is the leading cause of cancer death in children and adults under the age of 35 (Greenlee et al., 2000). Chronic myelogenous leukemia (CML) is one form that is highly resistant to most commonly used chemotherapeutic drugs including taxol (Bedi et al., 1995). The chimeric tyrosine kinase oncogene Bcr-Abl is the transforming activity responsible for CML (Daley et al., 1990). Recent studies on CML identified the atypical protein kinase C iota (PKC ι) as a critical downstream target of Bcr-Abl that is both necessary and sufficient to mediate the anti-apoptotic effects of Bcr-Abl (Murray and Fields, 1997; Jamieson et al., 1999). The mechanisms of PKC recruitment, regulation, and activation are unclear at present. Atypical PKCs are distinctly set apart from other members of the PKC superfamily by virtue of their characteristic N-terminal regulatory V1 domain, which comprises roughly the first 113 amino acids (Selbie et al., 1993). At least three proteins, p62, MEK5, and Par-6, have been found to functionally interact with the V1 domain (Moscat and Diaz-Meco, 2000). This places the V1 domain into a complex regulatory context and renders it an attractive target for pharmacological intervention. No three-dimensional structure of a V1 domain from any species is yet known. Here, we report on the rapid backbone chemical shift assignment of human PKC ι V1 and demonstrate the beneficial use of our new iterative semi-automated software IBIS.

*To whom correspondence should be addressed. E-mail: gerhard_wagner@hms.harvard.edu

Methods and experiments

The V1 domain (amino acids 1-113) of human PKC ι (NCBI accession number NP_002731) was produced as a GST fusion protein in *E. coli* BL21 cultured in LB or supplemented minimal R9 medium at 20 °C. R9 contains conventional M9 medium plus per liter 2 mg of thiamine, 0.1 mg of riboflavin, and 1 mg each of biotin, choline chloride, folic acid, niacinamide, pyridoxal, and pantothenic acid (Sigma). Cleavage with PreScission Protease (Amersham Pharmacia) affords a protein with five additional N-terminal residues (GPLGS). Accordingly, V1 residues in this paper are numbered from 1 to 118. Samples were typically concentrated to 1 mM in a final buffer of 20 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM DTT. The structural integrity of recombinant PKC ι V1 was confirmed by N-terminal protein sequencing and MALDI-TOF mass spectrometry.

After initial ^{1}H-^{15}N HSQC experiments, the following triple resonance experiments were carried out at 25 °C on [*U*-^{13}C; *U*-^{15}N]-V1 on Bruker Avance 500 and 600 spectrometers equipped with CryoProbes: HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB, HNCO, HN(CA)CO, and (H)CC(CO)NH-TOCSY (Ferentz and Wagner, 2000). Complete data acquisition was accomplished within one consecutive period of 140 h. The seven triple resonance experiments together with a high-resolution ^{1}H-^{15}N HSQC served as the complete and optimal input for our assignment program IBIS (see accompanying paper by Hyberts and Wagner, 2003). During manual inspection and when appropriate, information from a ^{15}N-edited NOESY-HSQC was used to confirm as-

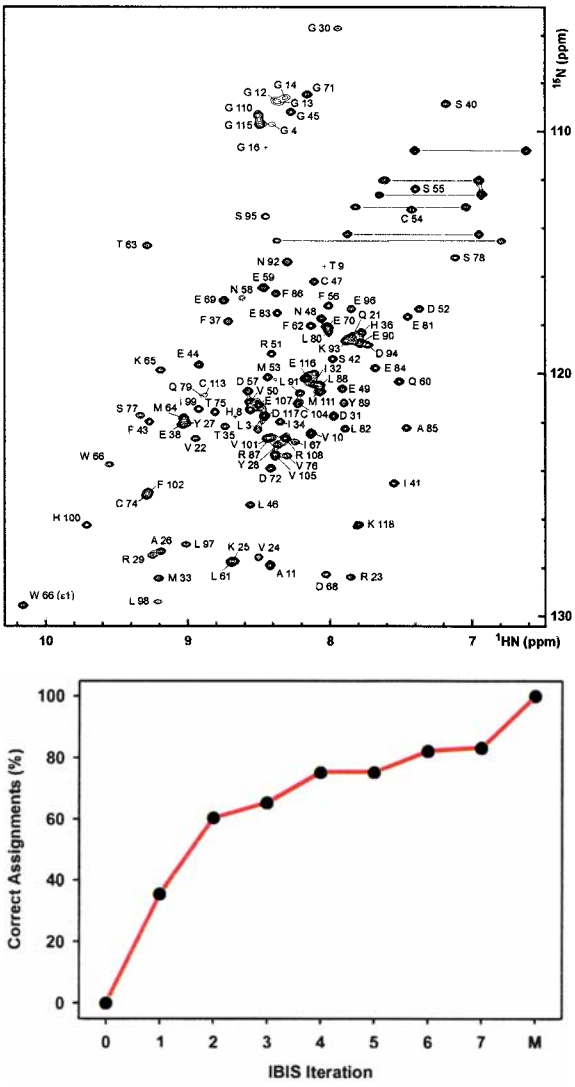

Figure 1. (A) The assigned ^{1}H-^{15}N HSQC spectrum of the V1 domain of human PKC ι acquired at 25 °C and pH 7.4. Resonances from side chain amines of three glutamines and three asparagines are connected by horizontal lines. Peak positions denoted by crosses lie just below the displayed contour level. (B) Percentage of correctly assigned residues as a function of IBIS iteration number. M denotes manual completion after seven IBIS iterations.

signments. Accurate chemical shift referencing and temperature calibration are critical for automated assignment procedures and were achieved with DSS and methanol standards, respectively. Data were processed with PROSA 3.7 (Güntert et al., 1992) and visualized with XEASY 1.3.13 (Bartels et al., 1995).

A total of seven iterative rounds of IBIS were performed within two working days. Manual analysis and completion of the assignment was then achieved readily (Figure 1A). Figure 1B illustrates the percentage of correctly assigned residues as a function of IBIS iteration.

In summary, we present here the application of our new semi-automated iterative assignment program IBIS to the rapid assignment of the V1 domain of PKC ι. We demonstrate that the task of experimental data acquisition, processing, and semi-automated complete backbone assignment can be achieved within less than ten days for a protein of 13.2 kDa.

Extent of assignment and data deposition

Complete assignments of ^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ, and ^{13}C' nuclei have been obtained for residues 2–14 and 21–118, except for eight prolines. For those residues, all the carbon resonances were assigned through correlations with subsequent amides in the (H)CC(CO)NH-TOCSY spectrum. Virtually all distal side chain carbons have also been assigned in these spin systems by virtue of the (H)CC(CO)NH-TOCSY spectrum. IBIS makes use of this experiment for tentative residue type identification during the assignment process. Residues 15–20 could not be assigned because no cross peaks are present in the ^{1}H-^{15}N HSQC, presumably due to conformational or amide proton exchange processes. Side chain protons were assigned manually since IBIS does not yet assign those resonances. The assignments of the V1 domain of human PKC ι have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-5661.

Acknowledgements

This research was supported by the National Institutes of Health (GM038608 and GM047467) and the MIT/Harvard Center for Magnetic Resonance (RR000995).

References

Bartels, C. et al. (1995) *J. Biomol. NMR*, **5**, 1–10.
Bedi, A. et al. (1995) *Blood*, **86**, 1148–1158.
Daley, G.Q. et al. (1990) *Science*, **247**, 824–830.
Ferentz, A.E. and Wagner, G. (2000) *Quart. Rev. Biophys.*, **33**, 29–65.
Greenlee, R.T. et al. (2000) *CA Cancer J. Clin.*, **50**, 7–33.
Güntert, P. et al. (1992) *J. Biomol. NMR*, **2**, 619–629.
Hyberts, S.G. and Wagner, G. (2003) *J. Biomol. NMR* **26**, 335–344.
Jamieson, L. et al. (1999) *J. Biol. Chem.*, **274**, 3927–3930.
Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep.*, **1**, 399–403.
Murray, N.R. and Fields, A.P. (1997) *J. Biol. Chem.*, **272**, 27521–27524.
Selbie, L.A. et al. (1993) *J. Biol. Chem.*, **268**, 24296–24302.