Journal of Biomolecular NMR, **27:** 87–88, 2003.
KLUWER/ESCOM

Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignments for the 21 kDa GTPase Rac1 complexed to GDP and Mg^{2+}

Roopa Thapar[a,b,*], Cathy D. Moore[c] & Sharon L. Campbell[a,b,*]
[a]*Department of Biochemistry and Biophysics and* [b]*Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, U.S.A.;* [c]*Pfizer Global R&D, San Diego, CA 92121, U.S.A.*

Received 16 January 2003; Accepted 7 April 2003

Key words: backbone assignment, GTPase, Rac1, Rho

Biological context

Rac1 is an important eukaryotic protein that belongs to the Rho family of Ras-related guanosine triphosphatases (GTPases). Rho family proteins function as binary molecular switches, shuttling between the active GTP-bound and the inactive GDP-bound forms (for a review, see Zohn et al., 1998). In the GTP-bound state, Rac1 activates several downstream targets and plays a critical role in regulating the cytoskeleton, activating gene transcription, controlling cell cycle progression, cell proliferation, and apoptosis. Crystal structures are available for the Rac1 F78S mutant bound to the GTP mimic GMPPNP (Hirshberg et al., 1997), Rac1 complexed to its regulators Tiam1 (Worthylake et al., 2000), Sptp (Stebbins & Galan 2000), Exos (Wurtele et al., 2001), RhoGDI (Grizot et al., 2001) and effectors Arfaptin (Tarricone et al., 2001) and p67phox (Lapouge et al., 2000). These X-ray structures reveal that regions in Rac1 that are important for molecular recognition can adopt different conformations when complexed to effectors, suggesting that functionally important regions are also conformationally mobile. Hence, our interest lies in elucidating the structural and dynamic properties of Rac1 that are important for effector recognition in solution. No solution structure of Rac1 either free or bound to an effector has yet been reported. We report herein the HN, ^{15}N, $^{13}C'$ and $^{13}C^{\alpha}$ sequence-specific resonance assignments of Rac1 complexed to GDP and Mg^{2+}.

*To whom correspondence should be addressed. E-mail addresses: thapar@email.unc.edu, campbesl@med.unc.edu

Methods and experiments

A C-terminally mutated form of Rac1, Rac1*dm*, (residues 1-188; C178S) was expressed in the vector pET21a (Novagen) and purified by cation-exchange chromatography followed by gel filtration. Sedimentation equilibrium experiments showed that the protein is monomeric at concentrations of 0.025–0.1 mM. However, a concentration dependent decrease in peak intensities (in going from 0.7 mM to 1.5 mM) was observed in 3D HNCA spectra, suggesting that the protein may self-associate at concentrations greater than 1 mM.

Rac1*dm* NMR samples contained 0.5–0.7 mM Rac1 in 50 mM Tris maleate buffer, 50 mM NaCl, 100 μM GDP, 20 mM $MgCl_2$, 10 mM DTT, 0.1% sodium azide, at pH 6.8 in H_20:$^{2}H_2O$ (90:10). Triple resonance NMR experiments suffered from poor sensitivity due to chemical exchange broadening of a number of residues that lie in the vicinity of the bound nucleotide. A combination of perdeuteration and residue-specific labeling of amino acids was found to be a useful assignment strategy. The following samples were used: a 0.7 mM perdeuterated and uniformly ^{15}N- and ^{13}C-labeled sample, a uniformly ^{15}N- and ^{13}C-labeled, 75% deuterated sample, selectively ^{15}N-labeled L,I,V,F,Y,A,G,G/S samples, and uniformly ^{15}N-labeled with selective lysine and arginine unlabeled samples.

NMR data were collected at 25 °C on Inova 600 and 800 MHz spectrometers equipped with either a 5 mm or 8 mm z-gradient triple resonance probe. The following experiments were used for assignments: 2D (^{15}N,^{1}H) TROSY, 3D versions of HNCA, HN(CO)CA, HNCO, CBCA(CO)NH, HNCACB, CC(CO)NH TOCSY, 3D (^{15}N, ^{1}H) NOESY-

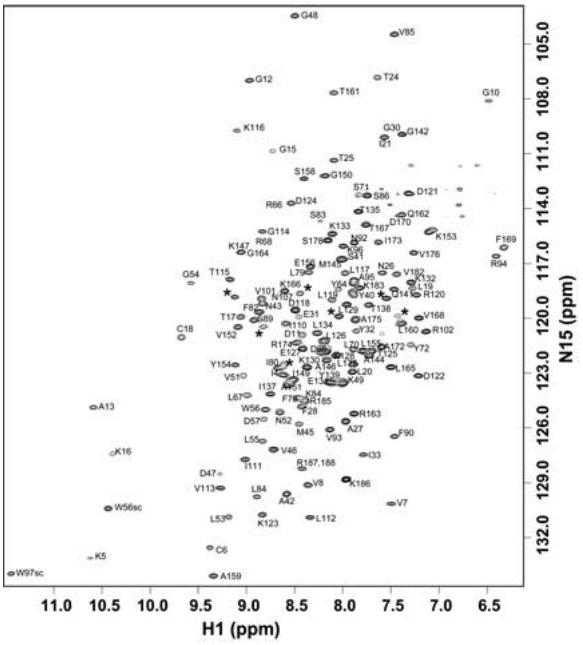

Figure 1. ^{1}H,^{15}N-TROSY spectrum of uniformly ^{15}N-labeled Rac1 (1-188; C178S) bound to GDP and Mg^{2+}, collected at a ^{1}H frequency of 600 MHz at pH 6.8 and 25 °C. The spectrum was collected with a Rac1 concentration of 0.5 mM with 256 increments and 16 scans per t_1 increment (total acquisition time is 2.5 h). The residue-specific assignments of the cross peaks arising from the backbone amide groups are indicated. Resonances marked with an asterisk remained unassigned. Unassigned cross peaks in the spectrum are side chain amide protons.

HSQC, and 3D (^{15}N, ^{1}H) TOCSY-HSQC. Most triple resonance experiments were collected using deuterium decoupling and either TROSY based ProteinPack sequences (Varian Inc., Palo Alto, CA) or those present in the Toronto-NMR package obtained from the ftp site of Lewis Kay, University of Toronto, Canada. Spectra were processed using the program Felix 98.2 (Accelrys).

Extent of assignments and data deposition

Cross peaks for 147 out of 173 possible resonances (188 residues minus 14 prolines and the N-terminal Met) are observed in the (^{1}H, ^{15}N) HSQC spectrum of Rac1*dm*.GDP.Mg^{2+} at pH 6.8 and 25 °C. Of these, backbone assignments for 140 (95% of 147 observable) resonances were completed. Resonances that are missing from the spectrum correspond to M1-A3, V9, V14, S22, Y23, T35-N39, V44, T58-D63, Q74-V77, A88, W97, H103-T108, E148, and I149. These residues lie near the GDP/Mg^{2+} binding site or in hinge regions and loops in the protein and most of these residues have been shown to be missing in NMR spectra of the Rac1 homolog Cdc42.GDP.Mg^{2+} (Feltham et al., 1997; Loh et al., 1999). In general, there is good agreement between the secondary structure predicted from the NMR data (based on the CSI, the pattern of NOEs, and the H-D exchange rates) and the crystal structure of Rac1. The greatest deviation from the crystal structure is for residues in β-strand 1 which are exchange broadened in the NMR spectra, and α-helix 3 which is predicted to be a coil by CSI with few of its backbone amides protected from amide exchange. Residues in switch-I, switch-II, α-helix 3 and the extreme C-terminus also exhibit lower than average ^{15}N-^{1}H heteronuclear NOE values. The HN, ^{15}N, $^{13}C'$ and $^{13}C^{\alpha}$ chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 5511.

Acknowledgements

The authors would like to acknowledge Dr Al Redfield for initiating high resolution NMR studies on Rac1. We are also grateful for his support and advice during the initial stages of the project. These studies were supported by NIH grant R01 CA83943-01.

References

Feltham, J.L., Dotsch, D., Raza, S., Manor, D., Cerione, R.A., Sutcliffe, M.J., Wagner, G. and Oswald, R.E. (1997) *Biochemistry*, **36**, 8755–8766.

Grizot, S., Faure, J., Fieschi, F., Vignais, P.V., Dagher, M.-C. and Pebay-Peyroula, E. (2001) *Biochemistry*, **40**, 10007.

Hirshberg, M., Stockley, R.W., Dodson, G. and Webb, M.R. (1997) *Nat. Struct. Biol.*, **4**, 147–152.

Lapouge, K., Smith, S.J.M., Walker, P.A., Gamblin, S.J., Smerdon, S.J. and Rittinger, K. (2000) *Mol. Cell*, **6**, 899.

Loh, A.P., Guo, W., Nicholson, L.K. and Oswald, R.E. (1999) *Biochemistry*, **38**, 12547–12557.

Stebbins, C.E. and Galan, J.E. (2000) *Mol. Cell*, **6**, 1449.

Tarricone, C., Xiao, B., Justin, N., Walker, P.A., Rittinger, K., Gamblin, S.J. and Smerdon, S.J. (2001) *Nature*, **411**, 215.

Worthylake, D.K., Rossman, K.L. and Sondek, J. (2000) *Nature*, **408**, 682.

Wurtele, M., Wolf, E., Pederson, K.J., Buchwald, G., Ahmadian, M.R., Barbieri, J.T. and Wittinghofer, A. (2001) *Nat. Struct. Biol.*, **8**, 23.

Zohn, I.M., Campbell, S.L., Khosravi-Far, R., Rossman, K.L. and Der, C.J. (1998) *Oncogene*, **17**, 1415–1438.