Journal of Biomolecular NMR, **23:** 337–338, 2002.
KLUWER/ESCOM

Letter to the Editor: Sequence-specific (^{1}H, ^{15}N, ^{13}C) resonance assignment of the N-terminal domain of the cyclase-associated protein (CAP) from *Dictyostelium discoideum*

Till Rehm[a], Chrystelle Mavoungou[a], Lars Israel[b], Michael Schleicher[b] & Tad A. Holak[a,*]
[a]*Max-Planck-Institute of Biochemistry, D-82152 Martinsried, Germany;* [b]*Adolf-Butenandt-Institute, Ludwig-Maximilians-University, D-80336 Munich, Germany*

Received 19 June 2002; Accepted 18 July 2002

Key words: adenylyl cyclase-associated protein, CAP, NMR assignments

Biological context

The cyclase-associated protein (CAP) of *Dictyostelium discoideum* is an actin binding protein that is involved in the microfilament reorganization at anterior and posterior plasma membrane regions (Gottwald et al., 1996). CAP was first isolated from *Saccharomyces cerevisiae* as a component of the adenylyl cyclase (Cyr1p) complex (Field et al., 1990) and the protein is believed to act as one of the bridging proteins that link nutritional response signaling and changes in the actin cytoskeleton (for a review see Hubberstey et al., 2002). *Dictyostelium* CAP is a bifunctional protein as well. While the actin sequestering activity has been localized to the carboxy-terminal 210 amino acids, the N-terminus seems to mediate this activity in a PIP_2-regulated manner (Gottwald et al., 1996). The amino-terminal domain has also been shown to localize the whole protein to the membrane (Noegel et al., 1999). In general *Dictyostelium* CAP follows the domain organization of all CAP-homologues as given by Gerst et al. (1992): it consists of an amino-terminal domain encompassing residues 1–215 and a carboxy-terminal domain encompassing residues 255–464, separated by a proline-rich linker domain of 39 residues.

Our NMR characterization of two amino-terminal constructs revealed a different domain structure though. Investigations including linewidth analysis, mass-spectrometry and sequencing proved the structured amino-terminal domain of *Dictyostelium* CAP to exclude a serine-rich stretch at the N-terminus and to encompass the 176 residues from positions 51–226.

*To whom correspondence should be addressed. E-mail: holak@biochem.mpg.de

Methods and results

The cDNAs encoding the amino-terminal 226 residues of CAP plus a C-terminal His-tag (CAP-N′Px) or the 176 residues from position 51–226 (CAPN151-678) were cloned into the *Nde*I and *Bam*HI restriction sites of the pT7-7 expression vector (Tabor, 1996). *E. coli* BL21 harboring the plasmids were grown at 30 °C (CAP-N'Px) resp. 37 °C to an OD_{600} of 0.6–0.8. For expression of the protein IPTG was added to a final concentration of 0.5 mM and cells were further incubated over night. After lysis and centrifugation in both cases the 100,000 × *g* supernatants were then purified on DE52 (Whatman) anion exchange, phosphocellulose (P11, Whatman) cation exchange, hydroxyapatite (Bio-Rad) and Ni-NTA (Qiagen) columns following standard procedures. The samples were finally concentrated in a Centriprep-10 concentrator (Amicon).

Uniformly ^{15}N-^{13}C and ^{15}N isotopically enriched protein samples were prepared by growing the bacteria in minimal media containing $^{15}NH_4Cl$, either with or without ^{13}C-glucose, respectively. For selectively enriched samples, defined media (Senn, 1987) were used that contained 100 to 800 mg l^{-1} of the isotopically enriched amino acids and all other amino acids.

The following samples were available in concentrations ranging from 0.8 to 1.2 mM at pH 7.3: Uniformly ^{15}N labeled CAP, as well as selectively ^{15}N-Ala, ^{15}N-Phe, ^{15}N-Gly, ^{15}N-Ile, ^{15}N-Lys, ^{15}N-Leu, ^{15}N-Val and ^{15}N-Gly/^{15}N-Ser labeled samples and a

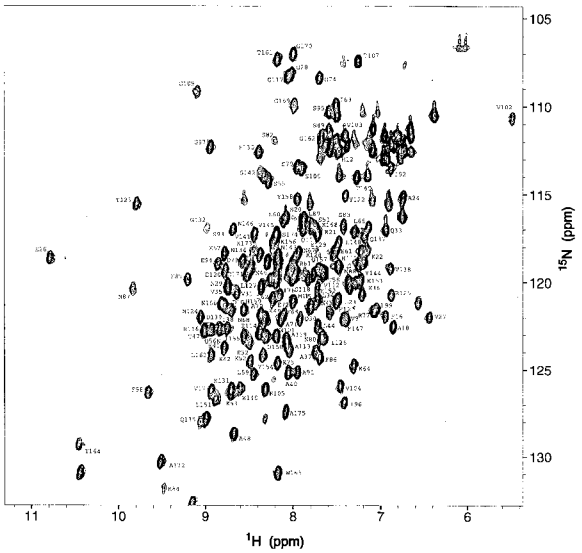

Figure 1. The ^{1}H-^{15}N-HSQC spectrum of CAP from *Dictyostelium discoideum* at 300 K and pH 7.3. The residue specific resonance assignment is indicated by the one letter amino acid code next to the corresponding signal; several are omitted for clarity.

^{15}N-^{13}C double labeled sample. All samples contained 10% D_2O. NMR spectra were recorded on Bruker DRX 600 and DMX 750 spectrometers equipped with triple resonance probeheads and pulsed-field gradient units. The backbone resonances were assigned using a pair of HNCA and CBCA(CO)NH triple-resonance spectra with the help of ^{15}N-HSQC spectra recorded from the selectively labeled samples. Furthermore a HNCO and two 3D ^{15}N-NOESY-HSQC spectra with mixing times of $\tau_m = 120$ ms and $\tau_m = 40$ ms and a ^{13}C-NOESY-HSQC with a mixing time of $\tau_m = 50$ ms were used. Assignment was accomplished using the software package *Sparky* (Goddard and Kneller, 2000).

Extent of assignment and data deposition

Figure 1 shows the ^{1}H-^{15}N-HSQC spectrum of CAP from *Dictyostelium discoideum*. Resonances of all backbone amide groups were assigned with the exception of the N-terminal residues S1 through L3, which could not be identified in the NMR spectra. Furthermore in the backbone 95% of H^{α} and 93% of C^{α} and C' were assigned as well as about half of the side chain atoms, including 95% of C^{β} and 81% of H^{β}. This assignment is sufficient to determine the structure of the protein and to analyze its dynamics. A table of the ^{1}H, ^{15}N, ^{13}C chemical shift assignment of CAP has been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the accession number 5393.

Acknowledgement

This work was supported by a DFG grant (SFB 413).

References

Field, J., Vojtek, A., Ballester, R., Bolger, G., Colicelli, J., Ferguson, K., Gerst, J., Kataoka, T., Michaeli, T., Powers, S., Riggs, M., Rodgers, L., Wieland, I., Wheland, B. and Wigler, M. (1990) *Cell*, **61**, 319–327.

Gerst, J.E., Rodgers, L., Riggs, M. and Wigler, M. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 4338–4342.

Goddard, T.D. and Kneller, D.G. (2000) 'SPARKY 3', University of California, San Francisco.

Gottwald, U., Brokamp, R., Karakesisoglou, I., Schleicher, M. and Noegel A.A. (1996) *Mol. Biol. Cell*, **7**, 261–272.

Hubberstey, A.V. and Mottillio E.P. (2002) *FASEB*, **16**, 487–499.

Noegel, A.A., Rivero, F., Albrecht, R., Janssen, K.P., Köhler, J., Parent, C.A. and Schleicher, M. (1999) *J. Cell Sci.*, **112**, 3195–3203.

Senn, H., Eugster, A., Otting, G., Suter, F. and Wüthrich, K. (1987) *Eur. Biophys. J.*, **14**, 301–316.

Tabor, S. (1990) In *Current Protocols in Molecular Biology*, Ausubel, F.A., Brent, R., Kingsten, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K., Eds., Greene Publishing and Wiley Interscience, New York, NY, pp. 16.2.1–16.2.11.