Journal of Biomolecular NMR, **20:** 291–292, 2001.
KLUWER/ESCOM

Letter to the Editor: Backbone resonance assignment of protease from Mason-Pfizer monkey virus

V. Veverka[a,*], H. Bauerová[b], A. Zábranský[b], I. Pichová[b] & R. Hrabal[a]
[a]*NMR Laboratory, Institute of Chemical Technology, Technická 5, CZ-166 28 Prague, Czech Republic*
[b]*Department of Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo náměstí 2, CZ-166 10 Prague, Czech Republic*

Received 15 March 2001; Accepted 12 April 2001

Key words: Mason-Pfizer monkey virus, NMR resonance assignment, retroviral protease

Biological context

In retroviruses, the structural proteins and enzymes are synthesized as polyprotein precursors. Late in the virus life cycle, as the particle is released, a process called maturation proceeds, in which the viral protease is cleaved off from polyproteins and then it releases the mature proteins from protein precursors. The proteolytic processing of polyproteins is essential for the infectivity of the virus.

Mason-Pfizer monkey virus protease (M-PMV PR) is encoded by an independent reading frame *pro* and is translated as a part of Gag-Pro (Pr 95) and Gag-Pro-Pol (Pr 180) precursors (Sonigo et al., 1986). Similar to the other retroviral proteases, M-PMV PR is an aspartyl protease containing the active site triplet Asp-Thr-Gly (Sonigo et al., 1986). In the active state it is a homodimer, which means that each monomer brings half of the active site to the molecule (Katz et al., 1994). The overall fold of all known retroviral proteases is quite conservative, despite rather large varieties in their amino acid sequences (Wlodaver et al., 2000). However, M-PMV protease exists in three active forms, which is an unusual feature within this family (Zábranský et al., 1998). While in vivo M-PMV PR occurs as 17 kDa and 13 kDa (per monomer) molecules, in vitro it undergoes further self-processing at the C-terminus of the 13 kDa form yielding a 12 kDa form with lower proteolytic activity, however, with unchanged specificity (Zábranský et al., 1998). To understand the influence of the C-terminal extension of M-PMV PR on its activity, we have started a structural study of the shortest form (12 kDa) by isotopically aided NMR spectroscopy. Here we present results of the first step, i.e. the assignment of NMR resonances of the backbone of a doubly labeled (^{13}C,^{15}N) 12 kDa form of M-PMV PR.

*To whom correspondence should be addressed. E-mail: Vaclav.Veverka@vscht.cz

Methods and experiments

Preparation of NMR sample

We have cloned a gene encoding the 12 kDa form of M-PMV PR into a bacterial expression vector (pBP-ATG12) containing an efficient IPTG inducible promoter of bacteriophage T7. To increase the stability of the protease, we have replaced two Cys residues (Cys 7 and Cys 106) by isosteric alanines (pBP-ATG12-C7/A,C106/A) (Andreansky et al., 1994). Protease was expressed in *E. coli* strain BL21(DE3). The cells were grown in a complete growth medium labeled with ^{13}C/^{15}N (Silantes, GmbH) and induced for protein expression by IPTG. Protease was accumulated into insoluble inclusion bodies and was isolated as described recently (Zábranský et al., 1998). Refolded protease was purified by ion-exchange chromatography (batch method) on QAE-Sephadex A 25 equilibrated with 50 mM Tris-HCl, pH 7.0, containing 2 mM EDTA. The purified protease was dialyzed against 50 mM acetate buffer pH 4.2 and concentrated by ultrafiltration (Amicon, membrane cutoff 10 000 kDa) to a final concentration of 1.0 mM. The concentration was determined either by the Bradford method or by UV absorption measurement (30 mM phosphate buffer, 6M guanidyl hydrochloride) using an extinction coefficient calculated from the primary structure of M-PMV PR (280 nm, 35420 $mM^{-1}cm^{-1}$).

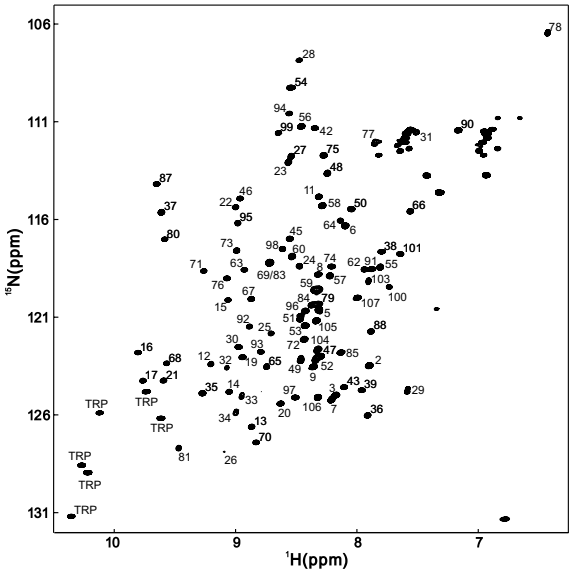

Figure 1. Two-dimensional ^{1}H-^{15}N HSQC spectrum of 1.0 mM Mason-Pfizer monkey virus protease at 37 °C. The assignments of the resonance peaks are indicated. Tryptophan indole resonances are marked 'TRP'.

Activity assay

The activity of the prepared enzyme was assayed by cleavage of the peptide substrate ATHQVYN-pHVRKA (Hrušková-Heidingsfeldová et al., 1995) followed by evaluation of the cleavage products by RP-HPLC on a Vydac C18 RP column in a methanol/H_2O system. The cleavage was carried out at 37 °C, 50 mM acetate buffer, pH 5.3. 10 μL of protease of known concentration was added to 100 μL of 0.5 mM substrate. The enzymatic reaction was interrupted by addition of 20% trifluoroacetic acid after 40 min.

NMR spectroscopy

NMR data were collected on a Bruker DRX500 Avance spectrometer at 37 °C using a triple-resonance probe equipped with three-axes actively shielded gradient coils. For all experiments Shigemi NMR tubes were used, allowing to reduce the final volume of the NMR samples to 250 μl. For the assignment of backbone resonances the following experiments were used: 2D ^{1}H-^{15}N, 3D CBCA(CO)NH, 3D HNCACB, 3D HNCA, 3D HN(CO)CA, 3D CC-TOCSY(CO)NH and 3D CC-COSY. NMR data were processed in the NMRPipe package (Delaglio et al., 1995) and assigned in NMRView (Johnson et al., 1994).

Extent of assignments and data deposition

Figure 1 shows the assigned ^{1}H-^{15}N HSQC spectrum of 107 amino acid residues, corresponding to each monomer. Complete assignment of the backbone resonances has been obtained for all non-proline residues. For the N-terminal residues Trp1, Asp18 and Lys102 as well as the proline residues only C_α and C_β resonances were determined from the CBCA(CO)NH experiment. From chemical shift index (CSI) analysis (Wishart et al., 1992) it turns out that the prevailing motif is an extended structure, a feature common among retroviral proteases (Wlodaver et al., 2000). Evidence was found for the following stretches: Trp1-Leu14, encompassing a part of the typical β-sheet located at the bottom of the molecular scaffold of retroviral proteases, two short stretches around the active site, i.e. Lys19-Thr27 and Val31-Lys35, respectively, and two more in the C-terminal half, i.e. Lys66-Asp72 and Ser77-Gly94. Another typical structural feature found within this family is a short α-helix located before the C-terminus, in case of M-PMV PR Arg95-Met101. The assignments have been deposited in the BioMagResBank databank (http://www.bmrb.wisc.edu) under accession code 4967.

Acknowledgements

This work was supported by the Grant Agency of the Czech Republic, Grant 203/00/1241 and Fogarty International Award TW00050.

References

Andreanský, M., Hrušková-Heidingsfeldová, O., Sedláček, J., Konvalinka, J., Bláha, I., Ječmen, P., Hořejší, M., Štrop, P. and Fábry, M. (1991) FEBS Lett., **287**, 129–132.

Delaglio, F., Grzesiek, S., Vuister, G., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Hrušková-Heidingsfeldová, O., Andreanský, M., Fábry, M. and Bláha, I. (1995) *J. Biol. Chem.*, **270**, 15053–15058.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Katz, R.A. and Skalka, A.M. (1994) *Annu. Rev. Biochem.*, **63**, 133–173.

Sonigo, P., Barker, C., Hunter, E. and Wain-Hobson, S. (1986) *Cell*, **45**, 375–385.

Vogt, V.M. (1996) *Curr. Top. Microbiol. Immunol.*, **214**, 95–131.

Wishart, D.S., Sykes, B.D. and Richards, F.M. (1992) *Biochemistry*, **31**, 1647–1651.

Wlodaver, A. and Gustchina, A. (2000) *Biochim. Biophys. Acta*, **1477**, 16–34.

Zábranský, A., Andreanský, M., Hrušková-Heidingsfeldová, O., Havlíček, V., Hunter, E., Ruml, T. and Pichová, I. (1998) *Virology*, **245**, 250–256.