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Letter to the Editor: Complete ^{1}H, ^{15}N and ^{13}C assignment of a recombinant mouse major urinary protein

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Biological context

In the urine of sexually mature male mice there is an ensemble of protein isoforms defined as Major Urinary Proteins (MUP) complex (Knopf et al., 1983). They belong to the lipocalin superfamily and share with other members of this family the capacity to bind hydrophobic molecules (Schwende et al., 1986), some of which are odorants. A possible role of the molecules bound by these proteins is territorial marking (Cavaggioni et al., 1987) and/or modulation of behavioural reactions among conspecifics (Mucignat-Carretta et al., 1998). On the other hand, the proteins as such play an important role in the reproductive cycle of these rodents by acting as pheromones. In fact, MUP, either associated or free of their natural ligands, are able to interact with receptors in the vomeronasal organ of female mice, priming hormonal and physiological responses (Mucignat-Caretta et al., 1995). The understanding of the yet unknown mechanisms associated to all these biological functions necessarily requires the knowledge of structural details of these proteins. Because of the difficulty to single out the various wild-type isoforms, we have cloned one of them using the methylotrophic yeast *Pichia pastoris* as host (Ferrari et al., 1997). Here, we present the first complete sequence-specific assignment of this recombinant MUP (rMUP: 18707 Da), which provides the basis for elucidating its solution structure and conformational dynamics.

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Methods and results

The recombinant MUP was expressed and purified as described elsewhere (Ferrari et al., 1997). To produce ^{15}N-enriched rMUP, yeast cultures were grown and induced in minimal salt medium containing 3.5 g/l ^{15}N-ammonium sulfate as the sole nitrogen source. For the preparation of ^{13}C/^{15}N-labelled protein, the minimal medium contained 3.5 g/l ^{15}N-ammonium sulfate as sole nitrogen source and, as carbon source, first 0.7% ^{13}C-glycerol in the growth stage and then 0.5% ^{13}C-methanol in the expression stage. The molecular weights of the enriched samples were determined by time-of-flight mass spectrometry with MALDI equipment.

The ^{13}C/^{15}N- and ^{15}N-enriched rMUP samples were prepared in 10 mM phosphate buffer (pH 7.2, H_2O:D_2O = 90:10, v/v) at protein concentrations ranging between 1.7 and 2.7 mM. All the NMR experiments were recorded at 308 K on Bruker DMX spectrometers operating at ^{1}H resonance frequencies of 499.87 and 600.13 MHz. The heteronuclear 3D NMR experiments acquired on the labelled rMUP and used for resonance assignments are listed in Table 1 (Supplementary material, to be obtained from the corresponding author). All experiments made use of pulsed field gradients for coherence selection and artifact suppression, and utilized gradient sensitivity enhancement schemes wherever appropriate (Muhandiram and Kay, 1994). Quadrature detection in the indirectly detected dimensions was obtained either by the States-TPPI or by the echo/antiecho method. Chemical shifts were indirectly referenced to DSS (Wishart et al.,

1995) to ensure consistency among all spectra. The spectral data were processed using the Bruker XWIN-NMR 1.3 software package; peak-picking and data analysis of the transformed spectra were performed using AURELIA 2.5.9 (Bruker). The final 3D matrices typically consisted of 1024 × 128 × 128 or 1024 × 128 × 256 real data points.

Sequence-specific assignment of the backbone atoms was achieved by following independent connectivity pathways. Starting with the HNCA and the HN(CO)CA experiments, the assignment of the amide atoms and α-carbons was obtained. The few ambiguities encountered were resolved by the HNCACB experiment. Next, the HNCO and (HCA)CO(CA)NH (Löhr and Rüterjans, 1995) experiments provided the carbonyl chemical shifts. In order to verify the carbonyl and C^{α} assignment, we carried out HCACO and H(N)CA,CO (Szyperski et al., 1995) experiments which correlate both carbon species with H^{α} and HN, respectively. Thereby, all the previous assignments were confirmed and the H^{α} resonances were obtained as well.

The chemical shift values of the backbone atoms and the C^{β} atoms, obtained from the HNCACB experiment, were used as anchor points for the aliphatic side-chain resonance assignments. The CC(CO)NH-TOCSY (mixing time 17.5 ms) and H(C)CH-TOCSY (mixing time 18.1 ms) experiments were used to identify the aliphatic carbon and hydrogen atoms in the amino acid side-chains. The assignment of a few residues, mostly long-chain like arginines or lysines, was hampered by spectral overlaps of the H^{β}, H^{γ} and H^{δ} resonances. Thus, the HBHA(CC)(CO)NH experiment was used to distinguish between H^{β} and all other aliphatic side-chain proton resonances.

To achieve the assignment of aromatic nuclei, a 3D (H)CB(CGC)CH-TOCSY experiment was recorded. This experiment provided direct correlations between the C^{β} resonance and both protons and corresponding carbons of the aromatic ring. The side-chain carboxylate carbon resonances were completed from the HCACO data. The side-chain amide resonances of the Asn and Gln residues, as well as the $N^{\epsilon}H$ groups belonging to the Arg residues were assigned via a combination of HNCACB and ^{15}N-edited NOESY-HSQC spectra (mixing time 120 ms). Finally, the $C^{\epsilon}H_3$ groups of the three methionines were identified, in an analogous manner, from ^{1}H-^{13}C HSQC and ^{13}C-edited NOESY-HSQC spectra (mixing time 100 ms).

Extent of assignments and data deposition

All ^{1}H, ^{15}N and ^{13}C backbone and side-chain resonances of rMUP have been assigned (at pH 7.2 and 308 K), except for the side-chain ^{13}C carbonyl resonance of E140 and the $^{13}C^{\delta}$ and $^{13}C^{\epsilon}$ resonances of F41. The sequence-specific assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database, accession number BMRB-4340.

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