Journal of Biomolecular NMR, **17:** 353–354, 2000.
KLUWER/ESCOM

Letter to the Editor: Sequence-specific NMR resonance assignments of the backbone atoms for the olfactory marker protein, OMP

Donna M. Baldisseri[a,*], Joyce W. Margolis[b], Philip A. Omotosho[b], Brian F. Volkman[c] & Frank L. Margolis[b]

[a]*Department of Biochemistry and Molecular Biology, and* [b]*Department of Anatomy and Neurobiology, University of Maryland School of Medicine, 685 W. Baltimore Street, Baltimore, MD 21201, U.S.A.*
[c]*Department of Biochemistry, University of Wisconsin, 433 Babcock Drive, Madison, WI 53706, U.S.A.*

Received 5 May 2000; Accepted 5 June 2000

Key words: heteronuclear NMR, olfaction, olfactory marker protein, OMP, sequence-specific assignments

Biological context

OMP, the olfactory marker protein, is an abundant cytoplasmic protein whose expression is restricted, almost exclusively, to mature olfactory neurons. Since its discovery nearly 30 years ago, OMP has been used as the definitive identifier of mature olfactory neurons (Margolis, 1972). The primary sequence of OMP is phylogenetically conserved across vertebrate species and is 55% identical from *Xenopus* to human (Rössler et al., 1998). However, OMP has no homologs in the databases and, until recently, its function in olfactory sensory neurons has been elusive. The recent generation of OMP-null mice has provided clear in vivo and in vitro evidence for the involvement of OMP as a novel participant in the olfactory signal detection/transduction cascade (Youngentob and Margolis, 1999). To study this at the protein structural level we have initiated an NMR study of isotopically labeled OMP to solve its solution structure. Herein we present the sequential assignments for the backbone atoms of rat OMP and its secondary structure, which represents the first NMR structural description for any protein involved in chemosensory transduction.

Materials and results

Uniformly ^{15}N- and ^{13}C,^{15}N-labeled rat OMP was expressed and purified as described previously except that either Bio-Express 1000 (U-^{15}N 96–99%; CGM-1000-N) or Bio-Express 1000 (U-^{13}C 97–98%, U-^{15}N 96–99%; CGM-1000-CN) (Cambridge Isotopes, Andover, MA) was used in the growth media for production of ^{15}N-OMP or ^{13}C,^{15}N-OMP, respectively. Samples of 1.5 mM ^{15}N-OMP and 1.8 mM ^{13}C,^{15}N OMP were prepared in 10 mM phosphate, 0.1 mM EDTA, 0.3 mM NaN_3 at pH 6.6 in 95% H_2O and 5% D_2O.

Spectra were recorded on Bruker DMX 600 and 750 MHz spectrometers at 37 °C. For H^N, N, C^α, H^α, CO backbone and C^β side-chain sequential assignments, the following heteronuclear NMR experiments were performed: 2D ^{1}H-^{15}N HSQC, 3D CBCA(CO)NH, 3D HNCACB, 3D HNCO, 3D HCACO, 3D HCA(CO)N and 3D HNHA (Clore and Gronenborn, 1993). In addition, NOE data from the 3D ^{15}N-NOESY-HSQC (τ_m = 150 ms), 3D ^{15}N,^{15}N-HMQC-NOESY-HSQC (τ_m = 100 ms) and 4D ^{15}N,^{13}C-NOESY (τ_m = 100 ms) were acquired to confirm the resonance assignments (Wüthrich, 1986).

All correlations observed in the ^{1}H-^{15}N HSQC of OMP have been sequence-specifically assigned (Figure 1). Of the 156 that are possible (163 residues − 7 Pro), 6 are not detected (M1, A2, G42, Q61, Q85 and N93) due, most likely, to exchange-broadening (Lian et al., 1994). The 3D CBCA(CO)NH and 3D HNCACB data sets were used to sequentially assign the C^α and C^β resonances. Due to (i) redundancies in the primary sequence, (ii) the presence of 7 Pro residues and (iii) the absence of 6 correlations in the HSQC, ambiguities in the assignment process were

*To whom correspondence should be addressed. E-mail: dbaldiss@umaryland.edu

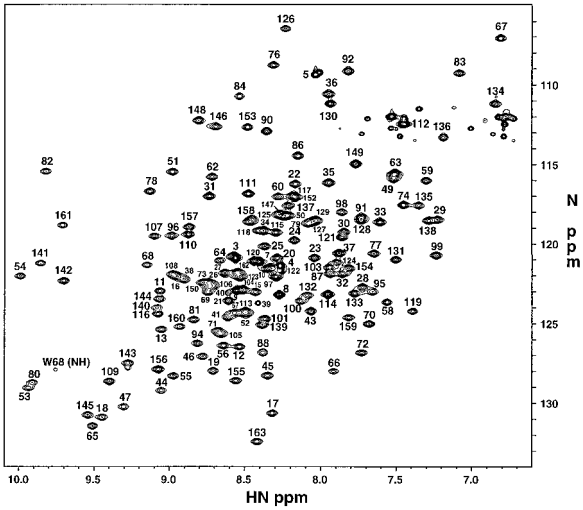

Figure 1. 600 MHz 2D ^{1}H-^{15}N HSQC of rat OMP labeled with the sequence-specific assignments for all detectable backbone amide correlations. Side-chain amide correlations are not assigned.

resolved either by the 3D HCA(CO)N data set or, in a few cases, by use of the 4D ^{15}N,^{13}C-NOESY.

Since the backbone assignments for rat OMP are essentially complete, the chemical shift index method (Wishart et al., 1997) was used, in addition to NOE patterns and coupling constants, to identify regions of extended secondary structure. Examination of these data identified 2 α-helices and 8 β-strands at residues Q10–D18 (β1), L21–Q34 (α1), E50–D56 (β2), N69–D73 (β3), G76–T83 (β4), L100–K109 (β5), A114–N118 (β6), L123–K134 (α2), V138–T144 (β7) and N153–Q162 (β8). Hydrogen exchange data (not shown) indicate that 25 highly conserved residues from β strands 1–4, 7 and 8 are protected from solvent and likely cluster to form the hydrophobic core of OMP.

Extent of assignments and data deposition

Work is in progress to extend the sequential assignments to the aliphatic side chain ^{1}H and ^{13}C atoms using the 3D H(CCO)NH and the 3D C(CO)NH datasets collected on triply labeled OMP and to the aromatic side-chain atoms using the 4D ^{13}C,^{13}C-NOESY experiment collected on doubly labeled OMP. The ^{1}H, ^{13}C and ^{15}N sequence-specific backbone resonance assignments reported here are located in BMRB-4735 at http://www.bmrb.wisc.edu and, along with side-chain assignments, are being used in the analysis of NOE data and dipolar couplings to obtain a high-resolution three-dimensional structure of OMP in solution.

Acknowledgements

NMR studies were carried out at the NMRFAM at Madison, WI with support from the NIH Biomedical Technology Program (RR02301) and additional equipment funding from the University of WI, NSF Academic Infrastructure Program (BIR-9214394), NIH Shared Instrumentation Program (RR02781, RR08438), NSF Biological Instrumentation Program (DMB-8415048) and the U.S.D.A. This work was supported, in part, by NIH DCD03112 (F.L.M.). We wish to thank Dr. D. J. Weber for helpful discussions.

References

Clore, G.M. and Gronenborn, A.M. (1993) *Determination of Structures of Larger Proteins in Solution by Three- and Four-dimensional Heteronuclear Magnetic Resonance Spectroscopy*, CRC Press, Boca Raton, FL.

Lian, L.Y., Barsukov, I.L., Sutcliffe, M.J., Sze, K.H. and Roberts, G.C.K. (1994) *Methods Enzymol.*, **239**, 657–700.

Margolis, F.L. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 1221–1224.

Rössler, P., Mezler, M. and Breer, H. (1998) *J. Comparative Neurol.*, **395**, 273–280.

Wishart, D.S., Watson, M.S., Boyko, R.F. and Sykes, B.D (1997) *J. Biomol. NMR*, **10**, 329–326.

Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, John Wiley, New York, NY.

Youngentob, S.L. and Margolis, F.L. (1999) *Neuroreport*, **10**, 15–19.