

^1H , ^{13}C and ^{15}N resonance assignments and second structure information of Gad m 1: a β -parvalbumin allergen from *Atlantic cod* (*Gadus morhua*)

A. H. Moraes · D. Ackerbauer · M. Kostadinova ·
M. Bublin · F. Ferreira · F. C. L. Almeida ·
H. Breiteneder · A. P. Valente

Received: 9 February 2012 / Accepted: 3 May 2012
© Springer Science+Business Media B.V. 2012

Abstract Gad m 1 is the major allergen from Atlantic cod. It belongs to β -parvalbumin protein family and is characterized by the presence of two calcium-binding sites so called EF-hand motifs. β -Parvalbumins such as Gad m 1 are the most important fish allergens and their high cross-reactivity is the cause of the observed polysensitization to various fish species in allergic patients. Despite extensive efforts, the complete elucidation of β -parvalbumin-IgE complexes has not been achieved yet. Allergen structural studies are essential for the development of novel immunotherapy strategies, including vaccination with hypoallergenic derivatives and chimeric molecules. Here, we report for the first time the NMR study of a β -parvalbumin: Gad m 1. This report includes: ^1H , ^{13}C and ^{15}N resonance assignments of Gad m 1 as well as the second structure information based on the ^{13}C chemical shifts.

Keywords Gad m 1 · β -parvalbumin · Allergen · NMR assignments · Secondary structure

Biological context

Allergenic proteins are able to stimulate an inappropriate IgE production in predisposed atopic individuals, which results in manifestations of clinical symptoms such as asthma, rhinitis and atopic dermatitis (Akdis 2006). Allergen immunotherapy is the only treatment that provides long-term clinical benefits. Allergen vaccine design includes the production of modified allergen molecules with a low-affinity for allergen-specific IgE (Wallner et al. 2007; Wild et al. 2007). Allergen structure determination is a crucial step in generating such molecules and also leads to an understanding of the molecular interactions between the allergen and IgE (Razzera et al. 2010) or the cells of the immune system.

β -Parvalbumins of fish represent one of the largest animal food allergen families, second only to tropomyosins of crustaceans and mollusks (Jenkins et al. 2007). Cross-reactions between β -parvalbumins from cod and other fish species are the cause of the frequently observed polysensitization to various species in fish allergic individuals (Griesmeier et al. 2010). This study focuses on the major allergen of cod, Gad m 1, which is a 108 amino acid residue protein and a member of parvalbumin protein family (Lewit-Bentley and Rety 2000; Breiteneder and Mills 2005). Eleven high-resolution structures of β -parvalbumins are available in the PDB, all of them were determined by X-ray crystallography. Gad m 1 is the first β -parvalbumin studied by NMR.

In this work, we present the sequence-specific ^1H , ^{13}C and ^{15}N resonance assignments and second structure

A. H. Moraes · F. C. L. Almeida · A. P. Valente (✉)
Centro Nacional de Ressonância Magnética, Instituto de
Bioquímica Médica, Universidade Federal do Rio de Janeiro,
Rio de Janeiro, Brazil
e-mail: valente@cnrmn.bioqmed.ufrj.br

D. Ackerbauer · M. Kostadinova · M. Bublin · H. Breiteneder
Department of Pathophysiology and Allergy Research, Medical
University of Vienna, Vienna, Austria

F. Ferreira
Christian Doppler Laboratory for Allergy Diagnosis and
Therapy, University of Salzburg, Salzburg, Austria

150 mM NaCl, pH 7.4. The fractions (5 mL) containing pure isotope-labeled rGad m 1 in SDS-PAGE analysis were pooled and concentrated as previously described.

To carry out the NMR assignment experiments, the samples (~64 μM—above this concentration the protein aggregates) of purified isotopically labeled (¹⁵N, ¹³C) Gad m 1 was prepared in 10 mM sodium phosphate, 0.5 mM CaCl₂ pH 7.0 containing 10% D₂O.

NMR spectroscopy

The NMR experiments were performed at 298 K using a Bruke Avance III 800 spectrometer equipped with a TXI 5 mm triple resonance probe and Bruker Avance III 600 equipped with 5 mm TCI triple resonance cryogenic-probe. The backbone assignment of the protein Gad m 1 by NMR spectroscopy was done through analysis of a collection of 3D triple-resonance experiments: HNCACB, HNCO, HNCA, HNCACO, CBCA(CO)NH. Side-chain resonance assignments have been achieved using the following

experiments: HBHA(CBCA)(CO)NH and HCCH-TOCSY. The assignment was confirmed and completed with ¹⁵N-edited NOESY-HSQC (120 ms mixing time) ¹³C-edited NOESY-HSQC (120 ms mixing time). All spectra were processed using Topspin 2.1 and analyzed using CARA 1.8.4 (Keller and Wulthrich 2004).

Assignments and data deposition

The ¹H–¹⁵N HSQC spectrum of Gad m 1 pH 7.0 at 298 K is shown in Fig. 1. The Gad m 1 spectrum presented NMR resonances well dispersed and almost all atoms were assigned (98%). The only exceptions were residues A2 and F3, which did not present ¹H–¹⁵N HSQC resonance signals and HE and CE1 of H27. Amide hydrogen NMR signals of G5, D9, E22 and A73 presented low intensities and their assignments were obtained by 3D triple-resonance experiments. The chemical shifts have been deposited in the BioMagnRes- Bank (<http://www.bmrb.wisc.edu>) under the accession number 18113.

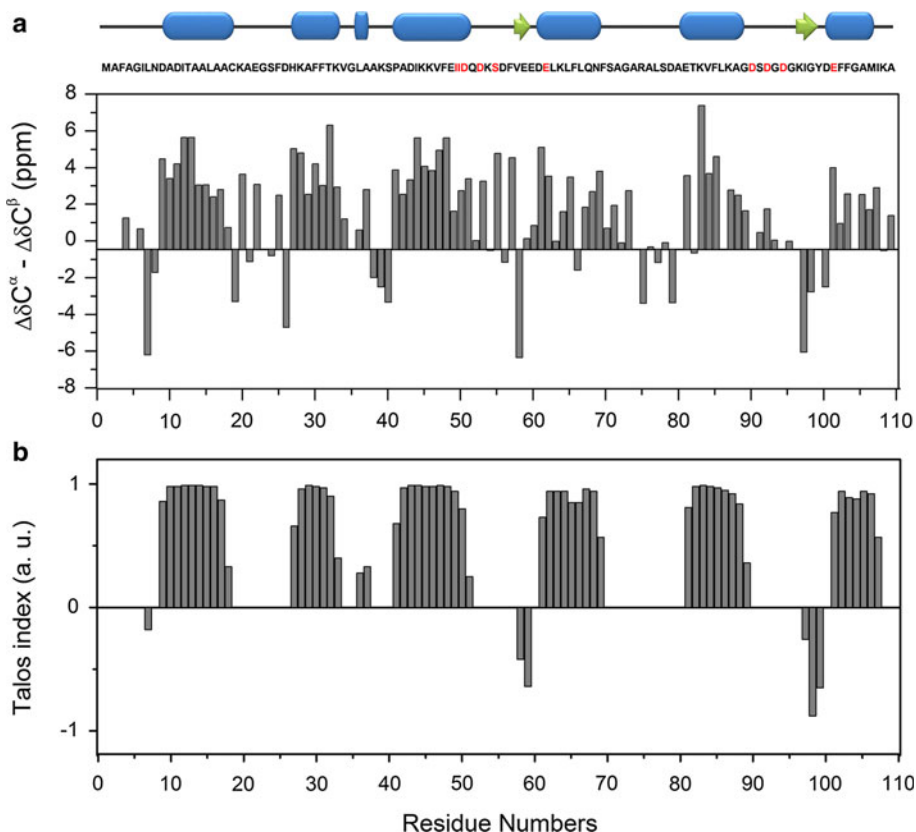


Fig. 2 Secondary structure information of Gad m 1. **a** Deviation of experimental chemical shifts from random coil values. Difference in chemical shift between C^α and C^β carbons for each residue (see “Methods”). The regions that presented positive values for these variations are identified as α -helix and negative as β -sheet. **b** The Talos index for secondary structure, which is consistent with the

prediction presented in **a**. The *upper panel* shows the secondary structure representation mapped by both analyses, where *barrels* and *arrows* represent regions of α -helix and β -sheets, respectively. Based on carp β -parvalbumin (1cdp), residues of the putative calcium-binding site are highlighted in red

Secondary structure information from the ^{13}C chemical shifts

The identification of the secondary structure was obtained through the analysis of the chemical shift deviations of the C^α ($\Delta\delta\text{C}^\alpha$) and C^β ($\Delta\delta\text{C}^\beta$) from the random coil values and using the program Talos+ (Shen et al. 2009). Figure 2a shows the values of $\Delta\delta\text{C}^\alpha$ minus $\Delta\delta\text{C}^\beta$ obtained for each residue and panel b shows the results obtained with the program Talos+. Positive and negative values of $\Delta\delta\text{C}^\alpha$ minus $\Delta\delta\text{C}^\beta$ and of the Talos index are associated with α -helix and β -sheet secondary structure, respectively. The Talos index indicates that residues 9–18, 27–33, 41–50, 61–69, 81–89 and 101–107 were involved in α -helix secondary structures, residues 36 and 37 also showed a positive index with low confidence. Interestingly, our results indicated that residues 56–57 and 97–99 were forming small β -sheets. These regions correspond to the putative calcium-binding sites, present in parvalbumin family members as an EF-hand motif (Fig. 2a). These features were only reported in the β -parvalbumin from carp (Ma et al. 2008). The NOEs analysis and structure calculation will confirm these suggestions.

References

- Akdis C (2006) Allergen and hypersensitivity mechanism of allergic disease. *Curr Opin Immunol* 18:718–726
- Breiteneder H, Mills E (2005) Molecular properties of food allergens. *J Allergy Clin Immunol* 115:14–23
- Griesmeier U, Vázquez-Cortés S, Bublin M, Radauer C, Ma Y, Briza P, Fernández-Rivas M, Breiteneder H (2010) Expression levels of parvalbumins determine allergenicity of fish species. *Allergy* 65:191–198
- Jenkins A, Breiteneder H, Mills N (2007) Evolutionary distance from human homologs reflects allergenicity of animal food proteins. *J Allergy Clin Immunol* 120:1399–1405
- Keller R, Wultrich K (2004) The computer aided resonance assignment. Available at: <http://www.nmr.ch>
- Lewit-Bentley A, Rety S (2000) EF-hand calcium-binding proteins. *Curr Opin Struct Biol* 10:637–643
- Ma Y, Griesmeier U, Susani M, Radauer C, Briza P, Erler A, Bublin M, Alessandri S, Himly M, Vázquez-Cortés S, de Arellano IR, Vassilopoulou E, Saxoni-Papageorgiou P, Knulst AC, Fernández-Rivas M, Hoffmann-Sommergruber K, Breiteneder H (2008) Comparison of natural and recombinant forms of the major fish allergen parvalbumin from cod and carp. *Mol Nutr Food Res* 52:S196–S207
- Razzera E, Gadermaier G, de Paula V, Almeida M, Egger M, Jahn-Schmid B, Almeida FCL, Ferreira F, Valente AP (2010) Mapping the interactions between a major pollen allergen and human IgE antibodies. *Structure* 18:1011–1021
- Shen Y, Delaglio F, Cornilescu G, Bax A (2009) Talos+: a hybrid method for predicting protein backbone torsion angle from NMR chemical shifts. *J Biomol NMR* 44:213–223
- Wallner M, Stöcklinger A, Thalhamer T, Bohle B, Vogel L, Briza P, Breiteneder H, Vieths S, Hartl A, Mari A, Ebner C, Lackner P, Hammerl P, Thalhamer J, Ferreira F (2007) Allergy multivaccines created by DNA shuffling of tree pollen allergens. *J Allergy Clin Immunol* 120:374–380
- Wild C, Wallner M, Hufnagl K, Fuchs H, Hoffmann-Sommergruber K, Breiteneder H, Scheiner O, Ferreira F, Wiedermann U (2007) A recombinant allergen chimera as novel mucosal vaccine candidate for prevention of multi-sensitivities. *Allergy* 62:33–41