Archives of Biochemistry and Biophysics 580 (2015) 93–101

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

The C-terminal domains of two homologous *Oleaceae* β-1,3-glucanases recognise carbohydrates differently: Laminarin binding by NMR

Héctor Zamora-Carreras [a], María Torres [b], Noemí Bustamante [a], Anjos L. Macedo [c], Rosalía Rodríguez [b], Mayte Villalba [b], Marta Bruix [a,*]

[a] Departamento de Química Física Biológica, Instituto de Química Física "Rocasolano", CSIC, Serrano 119, 28006 Madrid, Spain
[b] Departamento de Bioquímica y Biología Molecular I, Facultad de Química, Universidad Complutense, 28040 Madrid, Spain
[c] UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

ARTICLE INFO

Article history:
Received 13 May 2015
and in revised form 2 July 2015
Accepted 3 July 2015
Available online 4 July 2015

Keywords:
Fra e 9
Ole e 9
Allergy
Ash pollen
β-1,3-Glucanase
Carbohydrate-binding protein
NMR

ABSTRACT

Ole e 9 and Fra e 9 are two allergenic β-1,3-glucanases from olive and ash tree pollens, respectively. Both proteins present a modular structure with a catalytic N-terminal domain and a carbohydrate-binding module (CBM) at the C-terminus. Despite their significant sequence resemblance, they differ in some functional properties, such as their catalytic activity and the carbohydrate-binding ability. Here, we have studied the different capability of the recombinant C-terminal domain of both allergens to bind laminarin by NMR titrations, binding assays and ultracentrifugation. We show that rCtD-Ole e 9 has a higher affinity for laminarin than rCtD-Fra e 9. The complexes have different exchange regimes on the NMR time scale in agreement with the different affinity for laminarin observed in the biochemical experiments. Utilising NMR chemical shift perturbation data, we show that only one side of the protein surface is affected by the interaction and that the binding site is located in the inter-helical region between α1 and α2, which is buttressed by aromatic side chains. The binding surface is larger in rCtD-Ole e 9 which may account for its higher affinity for laminarin relative to rCtD-Fra e 9.

1. Introduction

Endo-β-1,3-glucanases are a family of glucan hydrolases (EC 3.2.1.39) widely distributed among plants, fungi and bacteria. They catalyse the hydrolytic cleavage of 1,3-D-glucosidic linkages in β-1,3-glucans and, on a larger scale, play a determining role in structural molecular conversions or metabolic functions [13,24] such as remodelling cell walls, cell expansion processes, cell–cell fusion, as well as cell division [39], microsporogenesis [43], pollen tube growth [40] or seed germination [7]. β-1,3-glucanases from higher plants belong to the glycosylhydrolase family 17 (GHF 17), whose components differ in molecular properties, cellular location and expression pattern. In addition to their constitutive biochemical functions, a defensive role has been attributed to these enzymes, as they can be expressed as a response to pathogens [26,27]. Based on these findings, they have been classified as the pathogen-related group 12 (PR-12). The antifungal activity of β-1,3-glucanases from higher plants has encouraged scientists to consider using molecular bioengineering to modify these enzymes with the goal of developing fungi-resistant crops [9].

Concerning their molecular structure, two main types of β-1,3-glucanases have been reported in plants: (i) long enzymes with 42–50 kDa molecular masses, composed by two domains – a large N-terminal domain (NtD, 33–40 kDa) with catalytic activity and a small C-terminal domain (CtD, around 10 kDa) with carbohydrate-binding capacity which has been called carbohydrate-binding module (CBM); and (ii) short β-1,3-glucanases (33–41 kDa) in which the CtD is lacking. The two domains from long β-1,3-glucanases fold independently and can be expressed recombinantly in heterologous systems. The three-dimensional modelling of all known NtD of β-1,3-glucanases closely resemble the canonical triose-phosphate isomerase (TIM)-barrel structure, although proteins from phylogenetically non-related species do not show high sequence identity.

In addition to the biochemical activity of β-1,3-glucanases, allergenic properties have been described for several members of this protein family. β-1,3-glucanases from pollens, fruits and natural latex (*Hevea brasiliensis*) are able to trigger allergic symptoms in hypersensitive patients. Ole e 9 and Fra e 9 are allergenic β-1,3-glucanases from olive tree (*Olea europaea*) and ash (*Fraxinus excelsior*) pollens, respectively; both species belonging to the *Oleaceae* family. The N- and C-terminal domains from Ole e 9 have been molecular and immunologically characterised [21]. Also, the

* Corresponding author.
E-mail address: mbruix@iqfr.csic.es (M. Bruix).

http://dx.doi.org/10.1016/j.abb.2015.07.004
0003-9861/

solution structure of the CtD-Ole e 9, a domain belonging to the CBM43 family, has been determined [37]. Moreover, we have recently reported the cloning, sequencing and the independently recombinant expression of the two domains of Fra e 9 which are composed of 320 residues for the NtD and 108 residues for the CtD [35]. The identity between rCtD-Fra e 9 and rCtD-Ole e 9 sequences calculated by the SIM alignment tool [20] is 55.7%, (Fig. 1A).

It has been postulated that CtD acts as to capture the β-1,3-glucan substrate which is then hydrolysed by the catalytic NtDs [6]. However, a detailed description of the process is still unknown. We expect that a high-resolution study of protein–ligand interactions would lead to a better understanding of the molecular basis of the biological recognition and functional mechanisms.

In this manuscript, we report the structural, hydrodynamic characterisation and the carbohydrate binding capability of rCtD-Fra e 9 from ash pollen in comparison with those of its olive tree pollen counterpart, Ole e 9, in order to understand its role in the catalytic process of long β-1,3-glucanases. The β-1,3-glucan selected for this study is laminarin from *Laminaria digitata*, a traditionally used oligosaccharide model [28,34].

2. Materials and methods

2.1. Materials

All ligands used for the binding assays were purchased from Sigma–Aldrich (USA): agarose, CM-cellulose (carboxymethyl cellulose), laminaritetraose (purity ⩾ 90%), laminarihexaose (purity ⩾ 99%), laminarin (from *Laminaria digitata*) and lichenan (from *Cetraria islandica*).

2.2. Protein production and purification

rCtD-Fra e 9, which comprises residues D354-S461 of Fra e 9, was produced in *Pichia pastoris* strain KM71 as previously described [29]. Briefly, cells were grown in 1 L of BMG (100 mM K_2HPO_4 pH 6, 0.34% yeast nitrogen base, 1% $(NH_4)_2SO_4$, $4 \cdot 10^{-5}$% biotin and 1% glycerol) for 72 h at 30 °C. Then, cells were grown in 200 mL of induction medium BMM (100 mM K_2HPO_4, pH 6, 0.34% yeast nitrogen base, 1% $(NH_4)_2SO_4$, $4 \cdot 10^{-5}$% biotin and 0.5% methanol). After 4 days, the supernatant was dialyzed in the presence of 20 mM NH_4HCO_3. Two chromatography steps: (i) gel filtration Sephadex G-50 column in 0.2 M NH_4HCO_3 and (ii) Nucleosil C-18 (RP-HPLC) TFA 0.1% with a gradient of acetonitrile (0–60%), were used for the protein purification. The purity was analysed by 15% SDS–PAGE. rCtD-Ole e 9 was produced and purified as previously described [37].

To produce ^{15}N–^{13}C labelled proteins, the same procedure was employed with the slight modifications described previously by Treviño et al. [36]; namely $(NH_4)_2SO_4$ was substituted by $(^{15}NH_4)_2SO_4$ (Cambridge Isotopes) in the BMG and BMM media, and 0.5% ^{13}C-glucose (Cambridge Isotopes) was used instead of glycerol in BMG and methanol was replaced by ^{13}C-methanol (Cambridge Isotopes) in BMM. All the samples were analysed by amino acid analysis, N-terminal sequencing and mass spectroscopy.

2.3. Carbohydrate-binding assay

The polysaccharide-binding activity of the rCtD from Fra e 9 and Ole e 9 was tested by affinity gel electrophoresis (AGE) as described previously [3]. Proteins (2 μg) were electrophoresed in native 15% polyacrylamide gels containing laminarin or lichenan

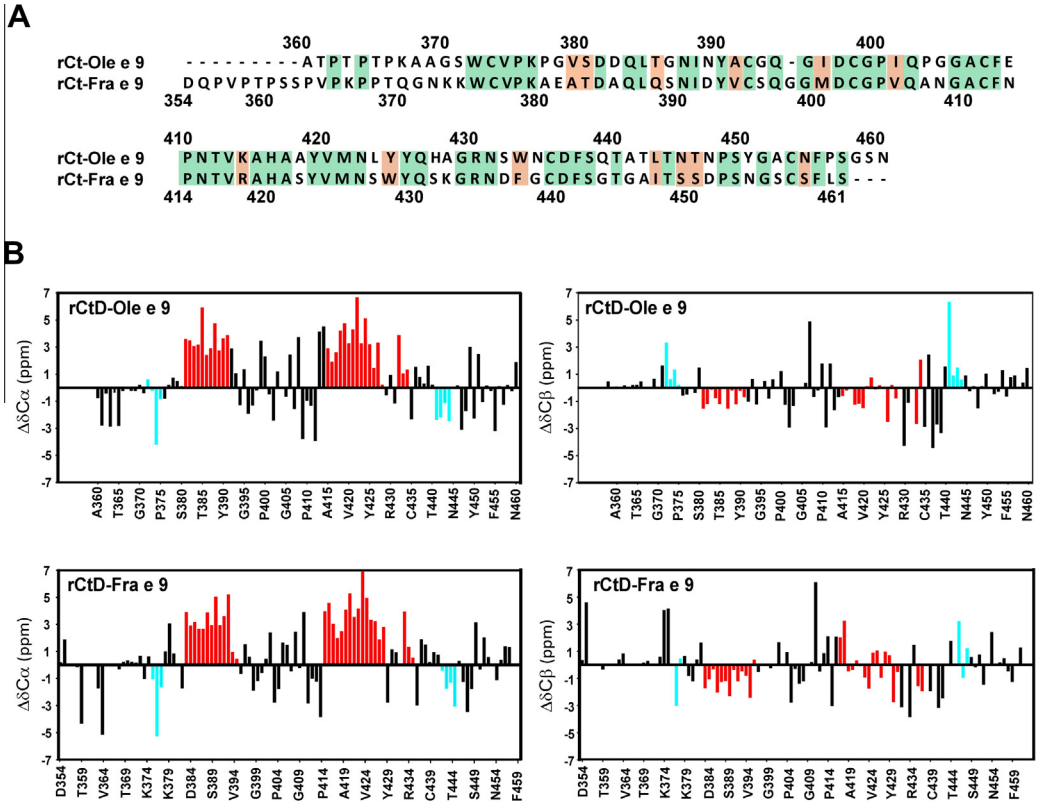

Fig. 1. Structural data of rCtD-Fra e 9 and rCtD-Ole e 9. (A) Sequence alignment. Identical residues are highlighted in green whereas similar residues are in orange. (B) $^{13}C_\alpha$ and $^{13}C_\beta$ conformational shift profiles for the CtD-Ole e 9 (top) [37] and rCtD-Fra e 9 (bottom). Regions with tendencies of secondary structure are coloured cyan for β-strands and red for α-helices; whereas loops are indicated in black.