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Original article

Oil bodies (oleosomes): Occurrence, structure, allergenicity

Oléosomes : présence structure, allergénicité

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Abstract

Oil bodies, or oleosomes, consist of lipid droplets confined within a protein-containing lipid monolayer that are widely distributed in oil seeds, particularly edible oil seeds. Amphiphilic proteins embedded in the lipid monolayer form an interface between the hydrophobic core of the oil bodies and the hydrophilic cytoplasm in which these organelles are distributed. They also play a crucial role in stabilizing oil bodies and preventing their coalescence via charge and steric hindrances. Oleosins, small proteins measuring 16–25 kDa, are the most abundant amphiphilic proteins occurring in the lipid monolayer surrounding the triglyceride core of oil bodies. They are composed of a hydrophobic α -helical hairpin anchored to the lipid monolayer and extended on both the *N*- and *C*-terminal ends by hydrophilic α -helical arms lying on the surface of the lipid monolayer in contact with the cytoplasm. Oleosins from peanut (Ara h 10, Ara h 11, Ara h 14, Ara h 15), hazelnut (Cor a 12, Cor a 13) and sesame (Ses i 4, Ses i 5) have been identified as major lipophilic IgE-binding allergens that are often associated with severe allergic reactions in sensitized subjects. Their allergenicity depends on the occurrence of IgE-binding epitopes along the hydrophilic arms attached to the hydrophobic α -helical hairpin. In addition, the phylogenetic relationships observed between oleosins belonging to different edible oil seeds suggest that some IgE-binding cross-reactivity may occur between oleosins of different origins. However, due to their lipophilic character, they are underrepresented in the water-soluble allergen extracts used in diagnosing food allergies and their role in triggering allergic responses in peanut and other tree nut allergies is thus doubtless greatly underestimated.

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Keywords: Oleosomes; Oil bodies; 3D-models; Hydrophobic cluster analysis (HCA); Oleosins; Caleosins; Steroleosins; Potential allergenicity; IgE-binding epitopes

Résumé

Les oleosomes sont des organelles constitués d'une gouttelette lipidique limitée par une monocouche de lipides associée à des protéines, largement répandus dans les graines, notamment dans les graines des oléagineux alimentaires. Les protéines amphiphiles insérées dans la monocouche lipidique jouent un rôle d'interface entre le contenu lipidique hydrophobe des oléosomes et le cytoplasme essentiellement hydrophile dans lequel ils sont dispersés. Ils jouent également un rôle stabilisateur des oléosomes en s'opposant à leur coalescence dans le cytoplasme. Les oléosines, protéines amphiphiles de 16–25 kDa, sont les protéines les plus abondantes de la monocouche lipidique limitant les oléosomes. Elles sont constituées d'un cœur hydrophobe de deux hélices- α disposées en épingle à cheveu, qui pénètre jusque dans le contenu lipidique de l'oléosome au travers de la monocouche lipidique et assure ainsi l'ancrage de la protéine dans l'oléosome. Le cœur hydrophobe se prolonge par deux bras *N*- et *C*-terminaux de structure α -hélicoïdale, hydrophiles, qui s'étalent à la surface de l'oléosome, au contact du cytoplasme. Les oleosines de l'arachide (Ara h 10, Ara h 11, Ara h 14, Ara h 15), de la noisette (Cor a 12, Cor a 13) et du sésame (Ses i 4, Ses i 5), sont des allergènes lipophiles majeurs, impliqués en particulier dans des cas d'allergies alimentaires sévères. L'allergénicité des oléosines est liée à l'existence d'épitopes liant les IgE,

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situés sur les deux bras hydrophiles exposés à la surface des oléosomes. En outre, les affinités phylogénétiques existant entre les oléosines d'origine différente, suggèrent la possibilité d'une réactivité croisée, notamment entre plusieurs graines d'oléagineux alimentaires. Néanmoins, les oléosines sont pratiquement absentes des extraits allergéniques utilisés dans le diagnostic des allergies alimentaires, en raison de leur liposolubilité. En conséquence, leur potentiel allergénique est très probablement sous-estimé.

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Mots clés : Oléosomes ; Corps lipidiques ; Modèles tridimensionnels ; Hydrophobic cluster analysis (HCA) ; Oléosines ; Potentiel allergénique ; Épitopes liant les IgE

1. Introduction

Triglycerides synthesized in higher plants are temporarily stored in small cellular organelles, the so-called oil bodies or oleosomes, which occur mainly in the cytoplasm of cotyledon cells of edible oil seeds, together with the larger protein bodies consisting of seed storage proteins. For many years, water-soluble proteins from the protein bodies were considered the exclusive source of allergens occurring in seeds. For example, Ara h 1, Ara h 2, Ara h 6 and Ara h 3, the major peanut (*Arachis hypogaea*) allergens, as well as Ara h agglutinin readily occur in seed protein bodies, whereas other water-soluble allergens such as Ara h 5, Ara h 8 and Ara h 9 are located in cytoplasm or secondarily migrate to the cell wall surrounding the cells (Ara h 9) [1]. It was only later that lipophilic allergens like oleosins and amphiphilic proteins exposed at the surface of oil bodies were suspected of potential allergenicity in previously sensitized subjects [2]. Later, different oleosins including Ara h 10, Ara h 11, Ara h 14 and Ara h 15 of peanut [3–5], Cor a 12 and Cor a 13 of hazelnut (*Corylus avellana*) [6,7], and Ses i 4 and Ses i 5 of sesame (*Sesamum indicum*) [8,9], were identified and characterized as major lipophilic allergens responsible for often severe allergic manifestations in allergic subjects. However, little is known about the occurrence, structure, allergenicity and potential IgE-binding cross-reactivity associated with oleosins from allergenic oil seeds and, in particular, relevant oleosin probes are still lacking in the essentially water-soluble protein extracts used for the laboratory diagnosis of food allergies. Herein we present a review of these apparently important allergens of which little is yet known regarding their structure and potential allergenicity.

2. Structure of oil bodies (oleosomes)

Oil bodies, also known as oleosomes, consist of lipid droplets that are widely distributed in oil seeds as a storage form of triglycerides within the cotyledon cells. In the cotyledon cells of peanut (*Arachis hypogaea*), they are small globule-shaped inclusions that remain unstained upon microscopic examination of ultrathin sections of the seeds after fixation and staining with water-soluble dyes (Fig. 1A). Under electron microscopy at higher magnification, they appear as small white globules of varying sizes arrayed between the larger heavily-stained protein bodies (Fig. 1B). Indeed, these organelles comprise a core made of hydrophobic triglycerides surrounded by a monolayer of phospholipids in which various amphiphilic proteins are embedded (Fig. 1C). The amphiphilic proteins consist chiefly of

oleosins and, to a lesser extent, of caleosins [10] and steroleosins [11]. These proteins, and especially oleosins, stabilize the oil bodies by forming an interface between the hydrophobic content of the oil body and the hydrophilic cytoplasm [12]. In addition, the abundance of oleosins in the lipid monolayer covering the oil bodies favours their dispersion in cells by hampering their coalescence via charge and steric hindrances.

3. Oleosins

Oleosins are small proteins of 16–20 kDa comprising a hydrophobic α -helical hairpin core anchoring the protein to the lipid monolayer surrounding the protein body. The hairpin loop penetrating the triglyceride core beneath the lipid monolayer contains two readily conserved Pro residues (Pro80 and Pro84 in Ses i 4). The α -helical hairpin is extended on both sides by two hydrophilic *N*- and *C*-terminal arms in which α -helical structures predominate (Fig. 1D). Both *N*- and *C*-terminal arms extend over the surface of the lipid monolayer and consist of the exposed part of the proteins in contact with the cytoplasm surrounding the oil bodies. The three-dimensional model built for sesame Ses i 4 oleosin resembles the first three-dimensional model proposed previously for peanut oleosins [13].

Other proteins anchored to the lipid monolayer of oil bodies consist of caleosins and steroleosins. Molecular modelling of caleosin from sesame (*Sesamum indicum*) seeds resulted in a 3D-model homologous to bacterial endo-1,4- β -xylanase constructed from two hydrophilic domains. The *N*-terminal domain contains a short α -helical hairpin enabling the caleosin to attach itself to the lipid monolayer of the oil body (Fig. 1E). Sesame steroleosin was similarly modelled from the X-ray coordinates of human corticosteroid 11- β -dehydrogenase and an insect apolipoprotein. The steroleosin comprises an α -helical hairpin-shaped signal sequence allowing the anchorage of the protein to the lipid monolayer, followed by a hydrophilic domain, which protrudes out of the lipid bilayer into the cytoplasm (Fig. 1F).

4. Phylogenetic relationships

Oleosins from edible oil seeds exhibit closely-related amino acid sequences, as seen in their multiple alignment (Fig. 2A). However, the most pronounced homologies occur at the central core of oleosins, corresponding to the α -helical hairpin, while both *N*- and *C*-terminal ends exhibit more variable amino acid sequences. As a result of these sequence homologies, oleosins from dicots cluster in two main groups of closely-related

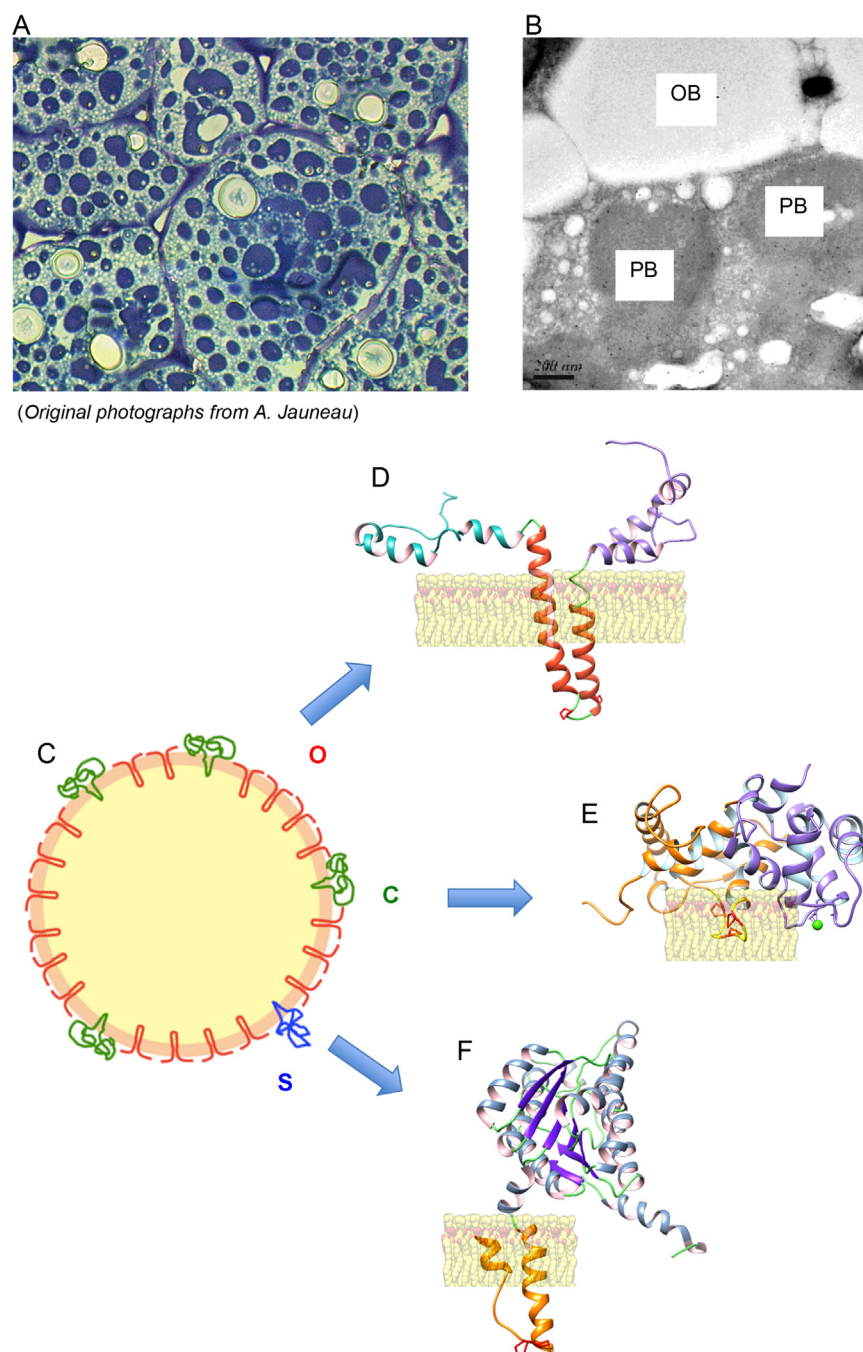


Fig. 1. A. Bright-field microscopy examination of toluidine blue-stained semi-thin section of peanut cotyledon cells showing a number of protein bodies (blue-coloured granulations) and oil bodies (unstained smaller granulations) distributed in cytoplasm. B. Electron microscopy of ultrathin section of peanut cotyledon cells showing oil bodies (OB) distributed among major allergen-containing protein bodies (PB). C. Cartoon showing the overall structure of an oil body, comprising a triglyceride core (coloured yellow) limited by a lipid monolayer (coloured orange) in which stabilizing amphiphilic oleosins (o), caleosins (c) and steroleosins (s) are embedded. D. Three-dimensional model built for oleosin Ses i 4, comprising a hydrophobic α -helical hairpin (coloured orange) anchored to the lipid monolayer (ML) and prolonged by two *N*- (coloured) and *C*-terminal (coloured) arms exposed at the oil body surface. E. Three-dimensional model built for sesame caleosin, consisting of two *N*- (coloured orange) and *C*-terminal (coloured violet) hydrophilic domains protruding on the surface of the lipid monolayer (ML). A short α -helical hairpin associated with the *N*-terminal domain provides anchorage of the protein to the lipid monolayer. F. Three-dimensional model built for sesame steroleosin, consisting of a hydrophobic α -helical-shaped signal sequence anchoring the protein to the lipid monolayer (ML), followed by a hydrophilic domain (coloured violet) located on the surface of the oil body. (Original photographs from A. Jauneau).

proteins, far from the monocot oleosins of wheat (*Triticum aestivum*) and maize (*Zea mays*), which cluster separately (Fig. 2B). In this respect, the first group consists of three clusters composed of oleosins from palm (*Elis guineensis*), sunflower (*Helianthus*

annuus) and castor bean (*Ricinus communis*) [1st group], peanut (Ara h 15) and soybean (*Glycine max*) [2nd group], and peanut (Ara h 11), sesame (Ses i 5), hazelnut (Cor a 13) and almond (*Prunus dulcis*) [3rd group], respectively. Oleosins in the second

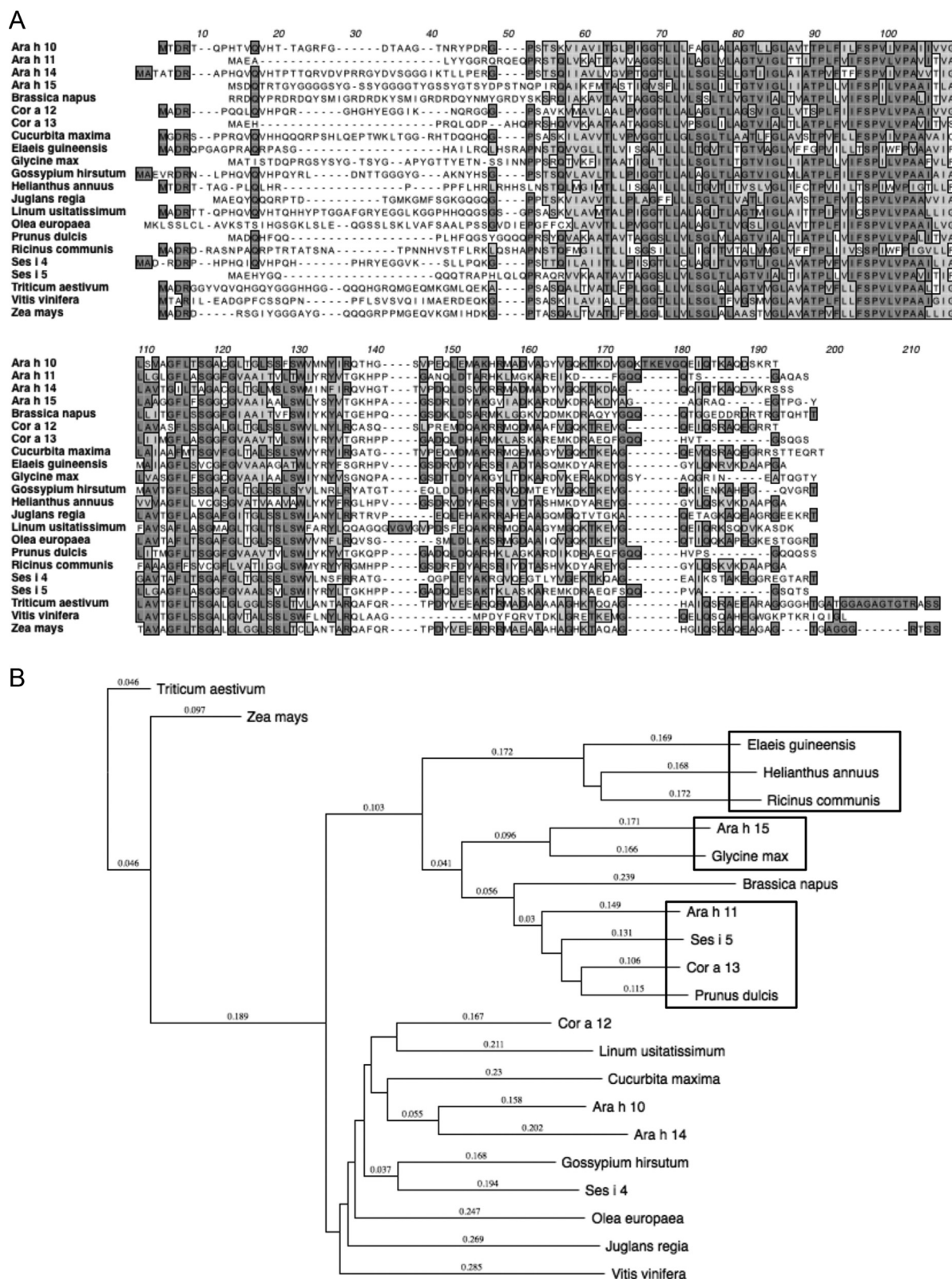


Fig. 2. A. Multiple amino acid sequence alignment of oleosins occurring in seeds used to prepare edible oils. Identical and homologous amino acid residues are deep grey and light grey boxed, respectively. Gaps are indicated by hyphens. B. Phylogenetic tree built up from the multiple amino acid alignment of oleosins. Seeds sharing closely-related oleosins are indicated by open boxes.

cluster are more distantly related. Obviously, these phylogenetic relationships may be associated with some cross-reactivities between oil seeds of different origin, e.g. between peanut and soybean, which both belong to the *Fabaceae* family.

5. Allergenicity

Oleosins from peanut (Ara h 10, Ara h 11, Ara h 14, Ara h 15), hazelnut (Cor a 12, Cor a 13) and sesame (Ses i 4, Ses i 5), have been identified and characterised as major lipophilic allergens responsible for frequently severe allergic manifestations in susceptible individuals, children and adults [2–9,14–16]. However, the role of these allergenic proteins in food allergies is probably underestimated since most allergen extracts used as diagnostic probes consist of water-soluble allergens and are practically devoid of lipophilic compounds like oleosins and other amphiphilic proteins from the lipid monolayer of oleosomes. Purified oleosin probes are urgently needed to enable more comprehensive diagnosis of food allergies like peanut, sesame and hazelnut allergies. In addition, depending on their phylogenetic relationships, cross-reacting allergenic oleosins from other edible oil seeds are probably involved in food allergies but they remain unrecognized in the absence of relevant diagnostic tools allowing specific identification of the corresponding IgE antibodies in the serum of allergic patients [7,15,16].

Predictive localization of IgE-binding epitopic regions along the amino acid sequence of Ses i 4 using the sophisticated hydrophobic cluster analysis (HCA) approach suggests the occurrence of three IgE-binding regions, all located on the hydrophilic N-terminal (1) and C-terminal (2) arms lying at the surface of the oil body (Fig. 3A and B). These IgE-binding regions correspond to exposed loops and α -helices in the three-dimensional model built for Ses i 4 (Fig. 3C). IgE-binding region #3 is homologous to another IgE-binding region previously identified in the peanut Ara h 15 oleosin allergen [16].

Due to their predominantly hydrophobic character, oil bodies are washed into the oil phase during cold-pressure treatment of edible oil seeds and become concentrated in the oil, in the absence of further refinement processes involving heating, solvent extraction and filtration. The high oleosin content of these first cold-pressed oils probably enhances their potential allergenicity and favours their involvement in triggering food allergies in susceptible individuals. Along this line, adulteration of olive oil by hazelnut oil has been identified as a possible risk for allergic consumers [14].

6. Bioinformatics

The amino acid sequences of oleosin allergens of *Arachis hypogaea* (accses. Q647G5 for Ara h 10, accses. Q45W87 for Ara h 11, accses. Q9AXI1 for Ara h 14, accses. Q647G3 for Ara h 15), *Sesamum indicum* (accses. Q9FUJ9 for Ses i 4, accses. Q9XHP2 for Ses i 5), *Corylus avellana* (accses. Q84T21 for Cor a 12, accses. Q84T91 for Cor a 13), and other oleosins from *Brassica napus* (accses. P29111), *Cucurbita maxima* (accses. XP_022993105.1), *Elaeis*

guineensis (accses. XP_010924186.1), *Glycine max* (accses. KRH52626.1), *Gossypium hirsutum* (accses. XP_016727497.1), *Helianthus annuus* (accses. XP_022038883.1), *Juglans regia* (accses. XP_018837699.1), *Linum usitatissimum* (accses. ABB01616.1), *Olea europaea* (accses. XP_022865502.1), *Prunus dulcis* (accses. XP_008234447.1), *Ricinus communis* (accses. XP_002511984.1), *Triticum aestivum* (accses. XP_020195411.1), *Vitis vinifera* (accses. XP_002273158.3) and *Zea mays* (accses. AAA68066.1) were taken from the NCBI protein database (<http://www.ncbi.nlm.nih.gov/pubmed>). Similarly, the amino acid sequences of sesame caleosin (accses. NP_001291323.1) and steroleosin (accses. NP_001291330.1) were taken from the NCBI protein database.

Multiple amino acid sequence alignment was performed with MacVector using the T-coffee programme [17]. An unrooted phylogenetic tree was built up from the multiple alignment of oleosins using the uncorrected neighbour-joining method of MacVector.

Hydrophobic Cluster Analysis (HCA) was performed to delineate the conserved secondary structure features along the amino acid sequences of oleosins, caleosins and steroleosins [18,19]. The HCA plots were generated at the RPBS web portal (<http://www.mobyle.rpbs.univ-paris-diderot.fr>).

Molecular modeling of the different segments of peanut (Ara h 10), sesame (Ses i 4) and hazelnut (Cor a 12) oleosins was performed with the YASARA structure program [20], using different proteins from the Protein Data Bank (PDB) [21] as templates. All of the modeled fragments were subsequently assembled and several steps of energy minimization in a water box were performed to improve the geometric and thermodynamic qualities of the three-dimensional models. Caleosin (accses.) and steroleosin (accses.) from sesame (*Sesamum indicum*) were homology-modelled similarly using as templates endo-1,4- β -xylanase A from *Bacillus subtilis* (PDB codes 1AXK [22], 2QZ3, 2DCZ [23] and 3EXU [24]), and both human (PDB codes 1XU9 [25], 3TFQ [26] and 4BB5 [27]) and murine (PDB code 4K26 [28]) corticosteroid 11- β -dehydrogenase isozyme 1, as well as locust (*Locusta migratoria*) apolipoprotein (PDB code 1AEP [29]), respectively. All the molecular cartoons were drawn with Chimera [30].

7. Discussion and conclusion

Obviously, oleosins and possibly other amphiphilic proteins associated with the lipid monolayer limiting oil bodies as stabilizing agents for these oil seed organelles, still remain poorly investigated with respect to their occurrence, structure and potential allergenic properties. This lack of investigation is due mainly on the difficulty in isolating oleosins from the corresponding oil bodies and properly analysing the physicochemical properties of these lipophilic proteins. However, their involvement in often severe allergic manifestations as major lipophilic allergens in hazelnut, peanut and sesame warrants accelerated research in this field and, in general, the rapid creation of relevant diagnostic tools. In this respect, the currently available water-soluble allergen extracts for diagnosis of food allergies, which

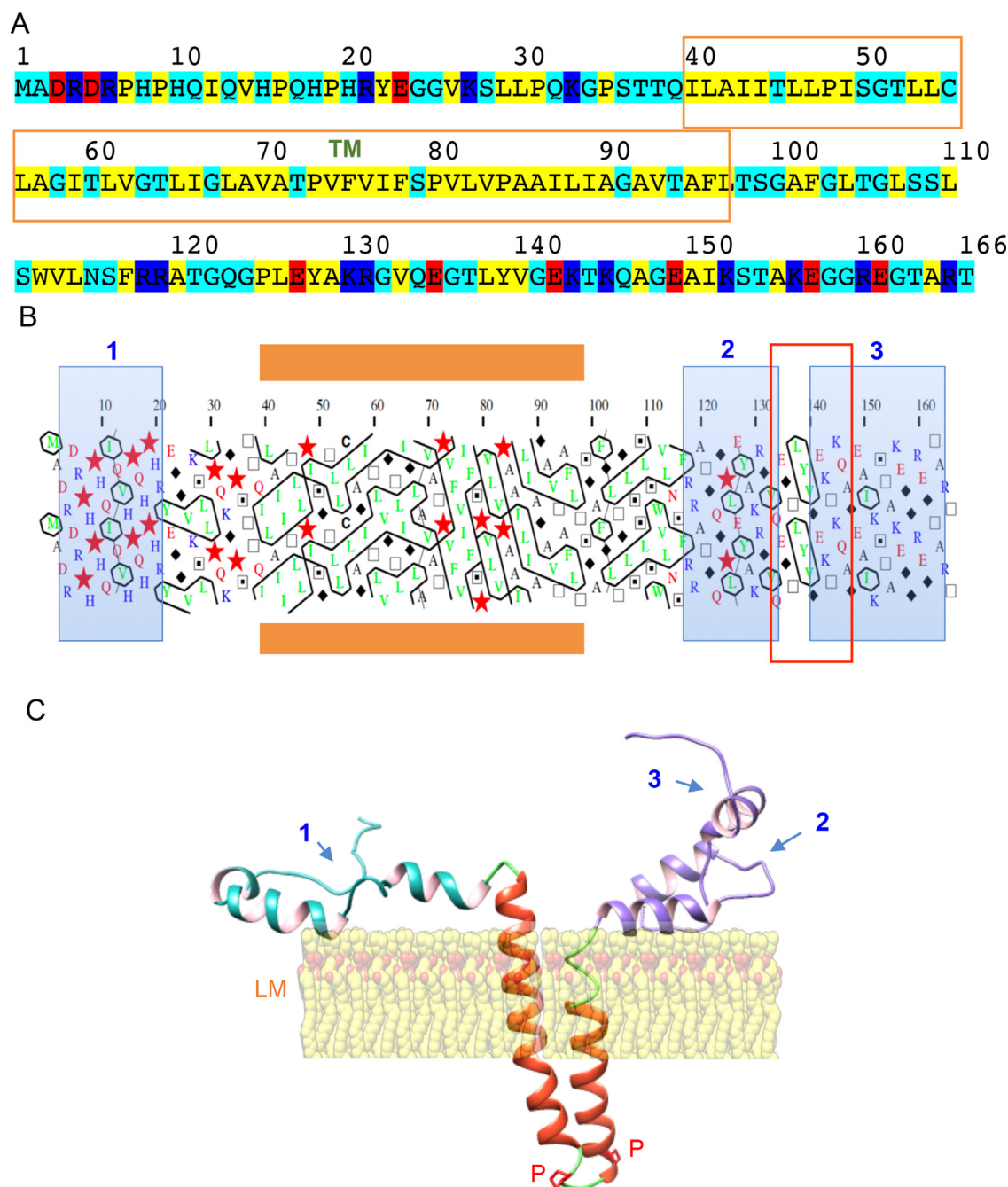


Fig. 3. A. Amino acid sequence of sesame oleosin Ses i 4. Polar (hydrophilic), non-polar (hydrophobic), negatively-charged (acid) and positively-charged (basic) amino acids are highlighted in cyan, yellow, red and blue, respectively. The hydrophobic α -helical core is orange boxed. B. Hydrophobic cluster analysis (HCA) plot of Ses i 4 showing the three predicted IgE-binding epitopic regions (numbered 1, 2, 3 and blue boxed) in the *N*- and *C*-terminal ends of the protein. The hydrophobic α -helical core is highlighted in orange. C. Three-dimensional model built for Ses i 4, showing the localization of the three predicted IgE-binding regions (1, 2, 3) at the *N*- and *C*-terminal ends of the protein. The lipid monolayer (LM) in which Ses i 4 is anchored is indicated.

are devoid of lipophilic proteins from oil bodies, are unable to correctly detect oleosin allergens [7].

The potential allergenicity of oleosins depends on the occurrence of IgE-binding epitopic regions located exclusively along the exposed *N*-terminal (one region) and *C*-terminal (two regions) arms connected to the hydrophobic α -helical hairpin anchoring oleosins to the lipid monolayer of oil bodies. Both hydrophilic (Ser, Thr, His) and charged (Asp, Glu, Arg, Lys) amino acid residues predominate in these epitopes.

In addition, some oleosins contain one (31NST of *Elaeis guineensis* oleosin, 31NSS of *Glycine max* oleosin, 23NTT of *Gossypium hirsutum* oleosin, 28NST of *Helianthus annuus* oleosin) or two (21NAT and 41NST of *Ricinus communis* oleosin) putative *N*-glycosylation sites that are sufficiently exposed to be actually glycosylated. This could introduce an additional source of allergenicity into these oleosins in the form of non-specific Cross-reactive Carbohydrate Determinants (CCD).

Although oleosins of oil seeds exhibit an extremely conserved hydrophobic α -helical hairpin core, their more variable N- and C-terminal arms are sufficiently conserved to allow oleosins of different origins to cluster in discrete groups in the phylogenetic tree built from their multiple amino acid sequence alignment (Fig. 3B). These phylogenetically related clusters suggest that oleosins of different origins could share some common allergenicity and possibly trigger some cross-reactions in sensitized people. In this respect, an IgE-binding epitope of peanut oleosin corresponding to the octapeptide SDQTRTGY has been shown to cross-react with buckwheat [4].

Disclosure of interest

The authors declare that they have no competing interest.

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