OLEOSINS - A NEW CLASS OF ABUNDANT SEED PROTEIN IN RAPESEED

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INTRODUCTION

Oleosins are a new class of hitherto unrecognised seed proteins, which constitute in excess of 20% of the total seed protein of mature rapeseed. Structurally and functionally related proteins have now been found in many other oilseed species, including maize, soybean, linseed, mustard, carrot, castor bean, sunflower and tobacco. Oleosins are a class of 19kDa amphipathic polypeptides, found exclusively at the oil:water interface of oil storage bodies in seeds. Oleosins were first purified from rapeseed by Murphy and Cummins in 1989(a). Since then, the biochemical and biophysical properties of oleosins and their molecular biology have been studied extensively in our laboratories.

SUBCELLULAR LOCALISATION OF OLEOSINS

Mature seeds of most cultivars of oilseed rape contain approximately 45% by weight oil and 25% by weight protein. The oil is encapsulated in small (0.3-0.5μm) diameter oil bodies, which tend to be arranged around the periphery of the embryo cells in the seed (Fig 1A). Towards the centre of these cells are arranged one or more large protein bodies. The protein bodies consist of aggregated complexes of the seed storage proteins cruciferin and napin. Cruciferin makes up approximately 45% total seed protein and napin approximately 20%.

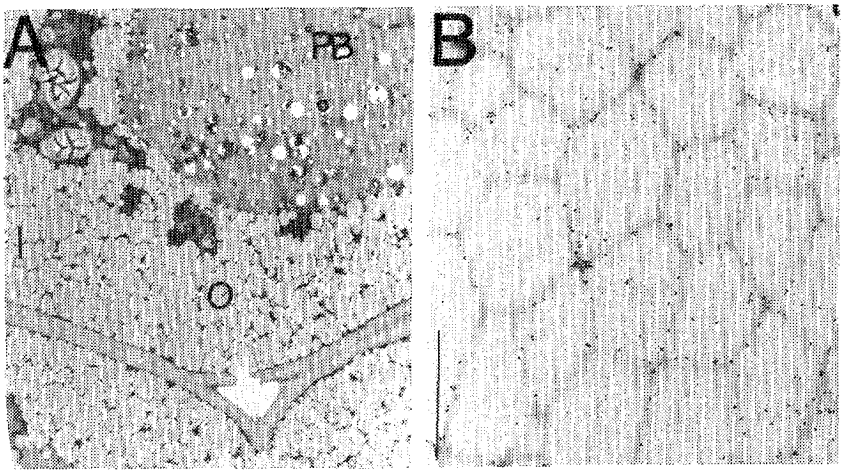

Figure 1
A, section of a cell from a mature seed showing the extensive oil body deposits (O), protein bodies (PB) and cell wall (CW). Bar is 1μm.
B, section of oil bodies in mature rapeseed labelled with anti-oleosin antibodies and visualised using a secondary antibody - colloidal gold conjugate (black dots). Bar is 1μm.

We found that the oil fraction of homogenised dry seeds from rapeseed contained substantial amounts of protein, which were highly enriched in a family of 19kDa polypeptides, the oleosins. These 19kDa polypeptides were not present in any other subcellular fraction from rapeseeds. The possibility that oleosins were secondarily associated with oil bodies during their isolation was discounted following immunogold labelling experiments, as shown in Figure 1B. Antibodies were raised against the purified 19kDa oleosins from rapeseed. These antibodies were then used to pinpoint the localisation of oleosins in sections of both developing and dry seed material. As can been seed in Figure 1B, the gold labels are distributed around the edges of the storage oil bodies. No gold labels were found to be associated with any other part of the seed cells. This finding was confirmed by the results of density gradient fractionation experiments, where all of the oleosins were shown to be located in the floating oil body fraction, rather than in any other membrane or protein fraction obtained from seed homogenates(Murphy et al, 1989).

OLEOSIN COMPOSITION

The amino acid and DNA compositions of the major rapeseed oleosins and their genes were elucidated by a combination of direct amino acid sequencing and by cloning and sequencing cDNAs. For direct amino acid sequencing, oleosins were purified by preparative SDS-PAGE, followed by electroblotting onto a PVDF membrane. The immobilised, blotted polypeptides were then sequenced using automated solid phase Edman degradation on a microsequencer. Full-length oleosins were found to be N-terminally blocked. For this reason, proteolytic fragments of 14-17kDa were generated using bacterial proteases prior to preparative SDS-PAGE. Oleosin cDNAs were obtained by screening an expression library in the phage λgt_{11} using monospecific antibodies prepared against purified oleosin proteins.

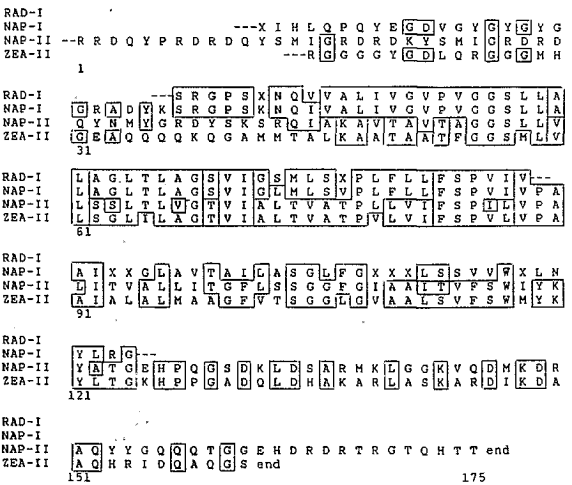

Figure 2
Partial amino acid sequences of two major oleosins of rapeseed (NAP-I, NAP-II), an oleosin from radish (RAD-I), and an oleosin from maize (ZEA-I). Identical residues are boxed.

The cDNAs were then subcloned and sequenced using conventional methods (Murphy et al, 1991). The nucleotide and amino acid sequences of the major rapeseed oleosins are shown in Figure 2, where the protein sequences are also compared with those of partial sequences from radish and maize (Vance & Huang, 1987) oleosins. As can be seen in this figure, all four oleosins show striking sequence homologies to one another. These sequence similarities are especially evident in the central hydrophobic domain of the oleosins, which constitutes a stretch of approximately 70 amino acid residues. In this large domain, no charged amino acids are present and only either non-polar or hydrophobic residues are found. This uninterrupted 70-residue hydrophobic domain is most unusual in any class of protein which has previously been studied. Intrinsic membrane proteins normally contain domains of approximately 25 relatively hydrophobic residues, which act as their transmembrane domains, but longer hydrophobic structures are very rare indeed.

STRUCTURE OF OLEOSINS

The primary sequence data for the rapeseed and other oleosins have been used to derive predictions concerning their possible secondary structures. The most striking structural motif shared by all oleosins sequenced to date is a highly conserved central region of about 70 uninterrupted hydrophobic/non-polar residues. We have used a variety of algorithms to predict the most likely structure to be adopted by oleosins, particularly in this interesting hydrophobic region. All of the algorithms that we have used give a strong prediction of ß-strand structure in this central hydrophobic region of the oleosins. This central region is predicted to be flanked by smaller amphipathic α-helical domains, as shown in Figure 3. The types of structures predicted to be adopted by oleosins from many different types of oilseed are all broadly similar to that of rapeseed as shown in Figure 3. One problem with the use of such algorithms is that they are based almost exclusively on data derived from the structures of soluble globular proteins. In the case of the oleosins, we are dealing with insoluble, interfacial proteins and one must therefore be cautious in extrapolating too readily from such predictive models. These models can, however, be useful in generating experimentally verifiable predictions and have been used with some success in predicting the amphipathic α-helical domains of mammalian apolipoproteins and the membrane spanning ß-sheet domains of bacterial porins (Murphy et al, 1991).

We have endeavoured to obtain direct experimental data on the structure of rapeseed oleosins using biophysical techniques, such as circular dichroism (CD) or Fourier-transfer infra-red (FT-IR) spectroscopy. Data from CD spectroscopic measurements indicate that native rapeseed oleosins contain approximately 22% of ß-strand structure and between 38% and 50% of α-helical structure. The amount of α-helical structure appears to depend on the molar ratio of fatty acid:protein in the oleosin preparation, with higher lipid protein ratios giving higher amounts of α-helical structure. These findings are somewhat at variance with the prediction shown in Figure 3, which is that there should be approximately 30% of ß-strand and only 19% of α-helical structure in the oleosin molecule. We conclude that the oleosins molecule may contain a relatively high ß-strand content as the lowest energy confirmation, when it is in a completely aqueous environment.

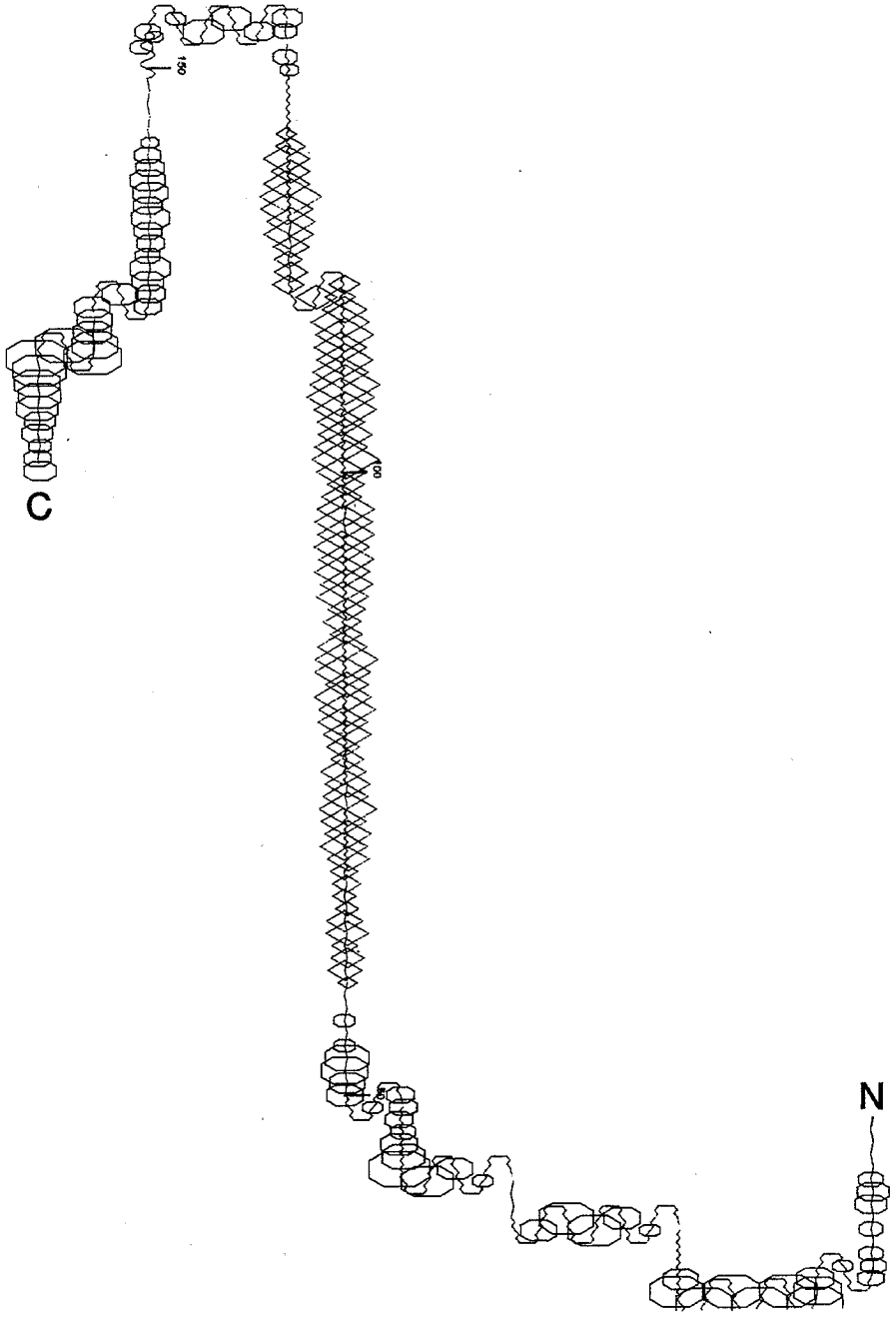

Fig 3 - Secondary structure prediction for the major oleosins from rapeseed. Diamond shapes represent hydrophobic/non-polar domains with high probability of ß-strand structure. Hexagonal shapes represent polar/charged domains with medium to high probability of α-helical structure. N,amino terminus; C, carboxy terminus.

A more biologically relevant situation is seen, however, when the protein is allowed to bind lipid, as would be the case when the oleosin is at a oil:water interface in the cell. Under such circumstances, the α-helical content of oleosins rises as more lipid becomes bound. This is similar to the lipid-induced rise in α-helical structure found in many animal apolipoproteins, fatty acid binding proteins and glutathione transferases. It is clear that more physical studies are required on the nature of oleosin lipid binding and on the secondary structure of oleosins before more definitive conclusions can be reached.

FUNCTION OF OLEOSINS

When oleosins were first discovered, it was proposed that they may play a role in the biosynthesis of storage oils in developing seeds. This possibility appears to be ruled out by our finding that oleosins are made several weeks after the accumulation of storage oil in developing rapeseed embryos(Murphy & Cummins, 1989b). On the other hand, it is known that the amount of oleosins in oleogenic plant tissues appears to be correlated with oil content. That oleosins play an important role in oil-storing plant tissues is evident from their great abundance. We suggest, therefore, that the function of oleosins is to provide a continuous monomolecular monobilayer around oil storage bodies in plants. We have found that the size of oil bodies appears to be determined by the quantity of oleosins synthesised during seed development(Cummins & Murphy, 1990). The oil bodies formed early in embryo development, at a time when no oleosins are present, tend to be very large (1-4 μm in diameter).

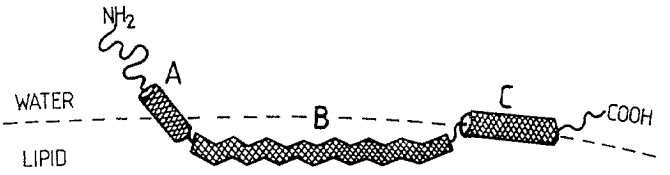

Figure 4
Proposed model of oleosins at the lipid:water interface of a storage oil body in plants.

a. N-terminal proximal polar α-helical domain. In the case of one of the rapeseed oleosins, this domain contains a sequence bearing some similarity to the lipoprotein lipase binding domain of human apolipoprotein CII. This domain may, therefore, be the site of attachment of the lipase responsible for oil body mobilisation.
b. Central 70-residue proline-rich hydrophobic domain. Due to its hydrophobic nature, this domain is probably immersed in lipid phase of the storage oil body.
c. C-terminal proximal, amphipathic α-helical domain. This contains a possible 4 x 11 residue tandem repeat, which has some similarities with the 11 residue tandem repeats found in all human and animal apolipoproteins sequenced to date.

Following oleosin synthesis, the oil bodies undergo a rapid decrease in size to approximately 0.3μm - a volume reduction of some 30-fold. The oleosin boundary layer may serve to render oil bodies inert and to preserve them during the drastic changes in moisture content that occur during seed maturation and subsequent germination.

Another possible role for oleosins is to provide a large surface area for lipase-mediated oil mobilisation following seed germination. Indeed, it has been suggested that oleosins may even act as receptors for lipase binding in a manner reminiscent of the binding of lipoprotein lipase to the apolipoprotein CII in mammalian transport lipid bodies (Murphy et al, 1991).

A model for the structure of oleosins and their orientation at the oil:water interface of oil-storage bodies is shown in Figure 4. It can be seen that approximately half of the oleosin molecule is embedded in the oil phase, while the other half of the molecule is accessible to the aqueous phase surrounding the oil bodies. One of the roles of oleosins appears to be as emulsifying agents acting to stabilise the very small oil droplets that exist in both wet and dry embryo cells in oilseeds. We are currently investigating the emulsifying properties of oleosins in a variety of physical systems. The fact that oleosins may have evolved specifically as highly active emulsifying agents over a period of many millions of years, may make them ideal components in long-lived emulsification systems.

Oleosins also represent a potential new source of nutritious protein in rapeseed. They constitute in excess of one fifth of total seed protein and, compared to the soluble seed storage proteins napin and cruciferin, oleosins are relatively lysine-rich. They also have the advantage that the oil fraction in which they initially partition is relatively free of potentially toxic compounds such as glucosinolates. It is therefore of interest to study both the potential biotechnological applications and the potential nutritional applications of oleosins, as these may add value to oilseed crops in the future.

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