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Oil bodies isolated from *Brassica napus* mature seed

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Plants store seed triacylglycerols in discrete lipid monolayer storage organelles called oil bodies. Only two proteins have been characterized from oil bodies, namely oleosin and caleosin, which are both integral membrane proteins. To better understand the protein composition this organelle, oil bodies were isolated from *Brassica napus* (cultivar westar) mature seed. Oil bodies were isolated using two published methods that utilize phase separation in aqueous media. Method 1 employed iterative washes in aqueous media containing sucrose, and 2M NaCl while method 2 made use of only one type of aqueous media (minus NaCl) put through multiple washes. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed method 1 to yield isolated oil bodies with higher purity based on the absence of the storage protein napin that was present in the total protein of isolated oil bodies from method 2. The oil bodies isolated with method 1 were subjected to washes in 2M NaCl or 8M urea to determine the nature of protein association to oil bodies. The isolated oil bodies were fractionated through petroleum ether to extract neutral lipids (triacylglycerols) that are contained by the monolayer membrane. Polar lipids were then extracted with chloroform/methanol. The interfacial pad which contained the associated proteins was suspended in water, sonicated, and subjected to acetone precipitation. Analysis of salt and urea washed oil body proteins by SDS-PAGE revealed abundant bands of the proper molecular weight for oleosins as well as at least 10 other additional proteins. Identification of these proteins by mass spectrometry will reveal novel proteins associated with oil bodies.