JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Constitution of Stable Artificial Oil Bodies with Triacylglycerol, Phospholipid, and Caleosin

MILES C. M. CHEN,† CHIA-LIN CHYAN,‡ TIGER T. T. LEE,† SHIN-HUNG HUANG,† AND JASON T. C. TZEN*,†

Graduate Institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan 40227, Republic of China, and Department of Chemistry, National Dong Hwa University, Hualien, Taiwan 974, Republic of China

Seed oil bodies are lipid storage organelles of 0.5−2 μm in diameter and comprise a triacylglycerol matrix shielded by a monolayer of phospholipids and proteins. These proteins include abundant structural proteins, oleosins, and at least two minor proteins termed caleosin and steroleosin. This study examined if artificial oil bodies (AOBs) composed of triacylglycerol and phospholipid could be stabilized by oleosin, caleosin, or steroleosin. Our results showed that stabilization effects could be realized by oleosin or caleosin but not by steroleosin. The sizes of the AOBs constituted with oleosin (0.5−2 μm) or caleosin (50−200 nm) were similar to or 10 times smaller than those of the native oil bodies. Recombinant caleosin expressed in *Escherichia coli* also encapsulated AOBs with a size, topology, and stability comparable to those encapsulated with native caleosin. A proteinase K digestion indicated that caleosin anchored the AOBs via its central hydrophobic domain of approximately 4 kDa. Isoelectrofocusing revealed that the isoelectric point of the caleosin-stabilized AOBs was pH 4.0. Aggregation of AOBs was observed at a pH lower than 4.5; thus, their stability and integrity were presumably contributed by surface caleosin via electronegative repulsion and steric hindrance. The caleosin-stabilized AOBs were thermostable up to 70 °C and potentially useful for biotechnological applications.

KEYWORDS: Artificial oil bodies; caleosin; oleosin; seed; stability

INTRODUCTION

Oily seeds store triacylglycerols (TAGs) as the energy for germination and subsequent seedling growth. The TAGs are assembled in discrete intracellular organelles termed oil bodies (*1*−*4*). An oil body contains a TAG matrix that is surrounded by a monolayer of phospholipids (PLs) embedded with proteins (*5*). These oil body-associated proteins include abundant structural proteins, oleosin isoforms, and some minor proteins (*6*, *7*). Two of the minor proteins have been separately identified and characterized as a calcium-binding protein named caleosin (*8*, *9*) and a sterol-binding protein named steroleosin (*10*). Oil bodies are remarkably stable in both cells and isolated preparations as a consequence of steric hindrance and electronegative repulsion provided by their surface proteins (*11*).

Oil bodies of diverse seeds consist of 94−98% neutral lipids (mainly TAGs), 0.5−2% PLs, and 0.5−3.5% proteins, and their average sizes range from 0.5 to 2 μm in diameter (*12*). Stable artificial oil bodies (AOBs) can be reconstituted with these three essential constituents in the proper proportions (*5*, *13*−*15*). AOBs as well as native oil bodies may serve as a useful assembly for diverse applications, e.g., as a vehicle for the production of recombinant proteins (*16*) and as a biocapsule for encapsulation of lactic acid bacteria in dairy products (*17*). Moreover, the oil matrix of AOBs may serve as an adequate carrier for hydrophobic molecules, such as neutraceutical compounds (e.g., antioxidant metabolites), pharmaceutical drugs (e.g., steroid hormones), and cosmetic lipids (e.g., healthy fatty acids). Without the presence of minor oil body proteins, oleosin or a mixture of oleosin isoforms has been demonstrated to encapsulate AOBs with comparable size and stability as native oil bodies (*18*). However, it has not been investigated whether other oil body-associated proteins, such as caleosin and steroleosin, can individually or cooperatively stabilize AOBs.

In this study, we examined if AOBs composed of TAG and PL could be stabilized by oleosin, caleosin, or steroleosin. A preliminary examination showed that stable AOBs could be encapsulated with oleosin or caleosin but not with steroleosin. Sizes of AOBs constituted with oleosin and caleosin were compared. AOBs constituted with recombinant caleosin were further characterized for their anchoring topology, isoelectric point, structural stability, and thermostability.

MATERIALS AND METHODS

Purification of Seed Oil Bodies and Oil Body Proteins. Mature seeds of sesame (*Sesamum indicum* L.) were gifts from the Crop Improvement Department, Tainan District Agricultural Improvement Station. The seeds were soaked in water for 10 min prior to the

* To whom correspondence should be addressed. Tel: 886-4-22840328. Fax: 886-4-22853527. E-mail: TCTZEN@dragon.nchu.edu.tw.
† National Chung-Hsing University.
‡ National Dong Hwa University.

10.1021/jf035533g CCC: $27.50
Published on Web 05/15/2004

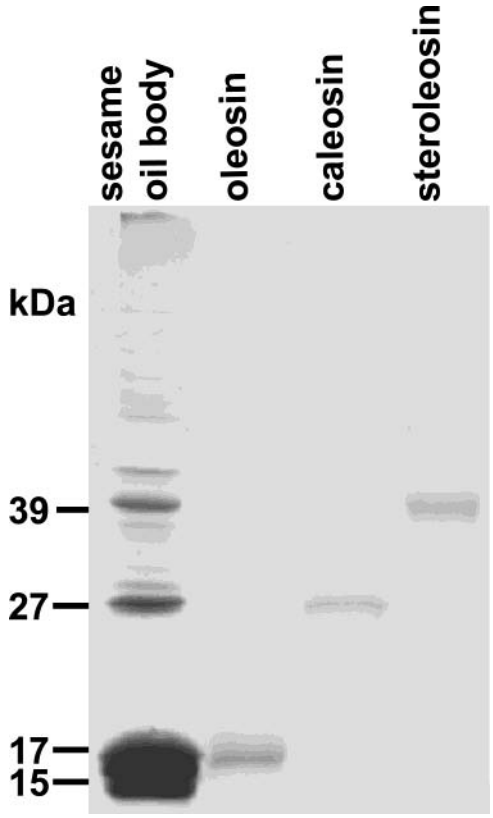

Figure 1. SDS–PAGE of purified oleosin, caleosin, and steroleosin from sesame oil body proteins. Labels on the left indicate the molecular masses of two oleosin isoforms (15 and 17 kDa), caleosin (27 kDa), and steroleosin (39 kDa).

purification of oil bodies according to the method developed by Tzen et al. (*6*). Oil body proteins, oleosin, caleosin, and steroleosin, were resolved in sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and eluted from gels according to the method described by Chen et al. (*7*).

Constitution of AOBs with Various Oil Body Proteins. For the constitution of AOBs, TAGs were obtained from sesame oil bodies by chemical extraction as reported previously (*5*), and dioleoyl phosphatidylcholine, the major PL found in oil bodies, was purchased from Sigma. AOBs were constituted with 15 mg of TAGs, 150 μg of PL, and 225 μg of oleosin, caleosin, or steroleosin in 1 mL of 10 mM sodium phosphate buffer, pH 7.5, according to the method described by Tai et al. (*14*).

Stability Test of Native Oil Bodies and AOBs. The stability was measured by turbidity changes with the time of a suspension of oil bodies in buffer. One milliliter of suspension mixture of native oil bodies or AOBs in 0.1 M sodium phosphate buffer, pH 7.5, was placed in a disposable 2 mL cuvette that was covered with Parafilm and kept undisturbed. The absorbance (A) at 600 nm of the suspension in the lower portion of the cuvette was recorded at time intervals in a Beckmen DU 530 spectrophotometer. The turbidity (T) of the suspension was proportional to 10^A, and the relative turbidity was expressed as $T/T_o = 10^A/10^{A_o} = 10^A/10^{2.0}$, where A_o was 2.0.

Microscopy of Purified Oil Bodies and AOBs. Purified oil bodies and AOBs constituted with oleosin or caleosin were suspended in 50 mM sodium phosphate buffer, pH 7.5, and observed under a Nikon type 104 light microscope. For electron microscopy, AOBs constituted with caleosin were fixed in 2.5% glutaraldehyde in 50 mM sodium phosphate buffer, pH 7.5, for 1 h and postfixed in 1% OsO_4 in the buffer for 2 h. Dehydration was carried out in a serial dilution of ethanol, and the samples were embedded in LR-White resin. Sections of 75 nm were stained with uranyl acetate and lead citrate and observed in a Hitachi H-300 electron microscope.

Overexpression of the Sesame Caleosin Clone in *Escherichia coli*. A full-length cDNA clone of sesame caleosin (accession no. AF109921) was constructed in the nonfusion expression vector, pET29a(+) (Novagen), using an *Nde* I site at the initial methionine position and a *Xho* I site in the polylinker of the vector (*8*). The recombinant plasmid was used to transform *E. coli* strain NovaBlue (DE3). Overexpression was induced by 0.1 mM IPTG in a bacteriophage T7 RNA polymerase/promoter system. Three hours after induction, the *E. coli* cells were harvested, crashed by sonication in 10 mM sodium phosphate buffer, pH 7.5, and then subjected to further analyses by SDS−PAGE and western blotting.

Antibody Preparation and Western Blotting. Antibodies against sesame caleosin were raised in chickens and purified from egg yolks for the immunoassay (*19*). In the immunoassay, proteins in an SDS−PAGE gel were transferred onto a nitrocellulose membrane using a Bio-Rad Trans-Blot system (Bio-Rad) according to the manufacturer's instructions. The membrane was subjected to immunodetection using secondary antibodies conjugated with horseradish peroxidase and then incubated with 3 mM 4-chloro-1-naphthol containing 0.015% H_2O_2 for color development.

Proteolytic Digestion of Caleosin in AOBs. Native caleosin (ca. 5 μg) purified from oil bodies or recombinant caleosin harvested from *E. coli* constituted in AOBs as described above was subjected to

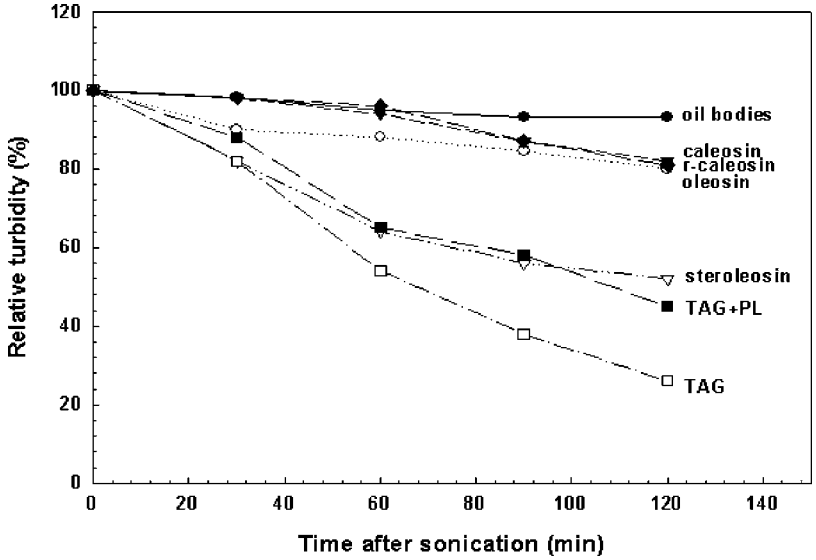

Figure 2. Turbidity tests for the stability of AOBs constituted with TAG, PL, and various oil body proteins. Along with TAG and PL, AOBs were constituted with oleosin, caleosin, recombinant caleosin (r-caleosin), or steroleosin. A suspension (1 mL) of sesame oil bodies or AOBs was placed in a 2 mL cuvette. The relative turbidity (T/T_0) at 600 nm of the lowest 0.5 mL of the suspension was measured at intervals.

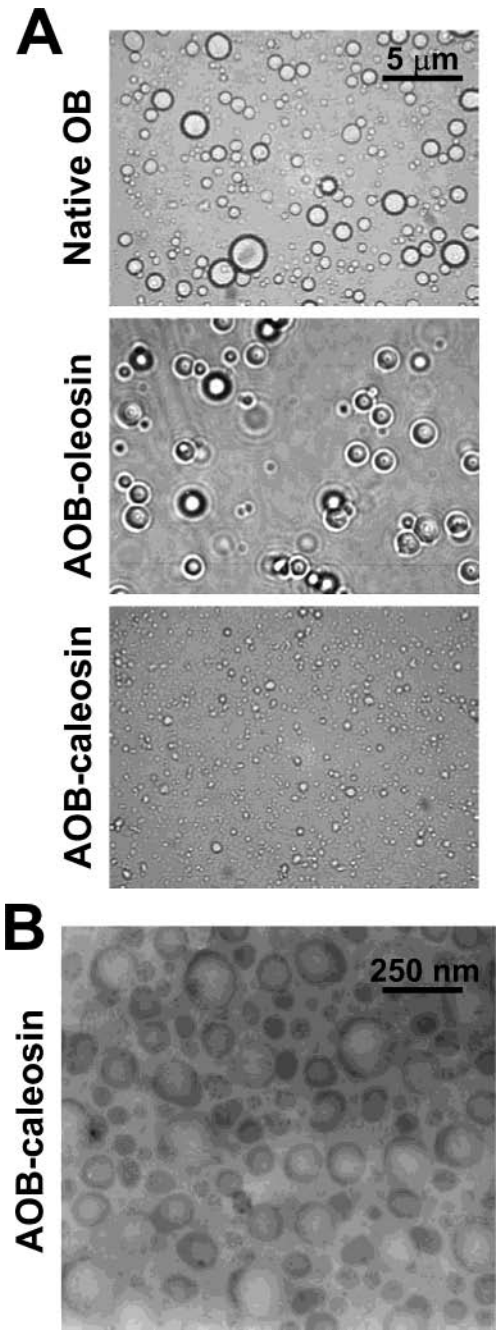

Figure 3. (**A**) Light microscopy of native oil bodies and AOBs constituted with oleosin or caleosin. Purified oil bodies and AOBs were suspended in the sodium phosphate buffer, pH 7.5, and observed under a light microscope. All three photos are of the same magnification. The bar represents 5 μm. (**B**) Electron microscopy of AOBs constituted with caleosin. AOBs constituted with caleosin were fixed, sectioned at 75 nm thickness, and observed under an electron microscope. The bar represents 250 nm.

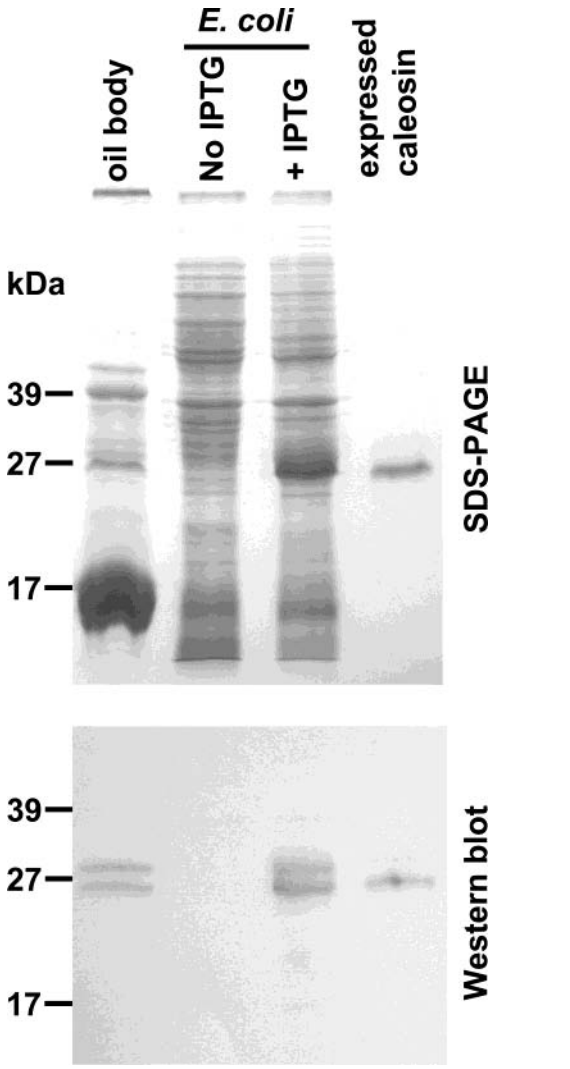

Figure 4. SDS–PAGE and western blotting of the overexpressed sesame caleosin in *E. coli*. Along with sesame oil body proteins (10 μg) and purified caleosin (1 μg), the total proteins (20 μg) of *E. coli* overexpressed in a nonfusion vector before or after IPTG induction were resolved in SDS–PAGE. A duplicate gel was transferred onto a nitrocellulose membrane and then subjected to immunoassay using antibodies against caleosin. Labels on the left indicate the molecular masses of oleosin, caleosin, and steroleosin.

proteolytic digestion using 200 ng of proteinase K (Sigma). The digestion was executed in a buffer containing 10 mM Tris-HCl, pH 8.0, and 10 mM $CaCl_2$ at 37 °C for 0, 1, 5, or 10 min (*20*). The degraded fragments were monitored in a 10% polyacrylamide Tricine SDS−PAGE gel.

Isoelectrofocusing of AOBs. Isoelectrofocusing was performed in a Bio-Rad Rotofor Cell (*21*). The horizontal cylindrical focusing cell of 55 mL was divided vertically into 20 chambers by partitions in order to minimize diffusion during electrophoresis and disturbance of the gradient during fractionation. Each partition was made of a monofilament polyester screen of 6 μm × 6 μm pore size. During electrofocusing, the Rotofor Cell rotated at 1 rpm and thus prevented the flotation of oil bodies. The fractionation of content in each chamber was achieved simultaneously within 1 s by a vacuum harvesting system. The pH gradient, made from 1% ampholyte of Bio-Lyte 3-10 (Bio-Rad), was preformed by applying an electric field at a constant power (12 W) for 20 min. AOBs (about 20 mg of TAGs) constituted with caleosin in 200 μL of 10 mM KCl suspension were applied to the gradient (at a position around pH 6) of the chamber. The same electric field was applied for 60 min to allow the focusing of the AOBs. The movement of the AOBs was observed visually. After fractionation, the AOBs and the pH along the gradient were detected at 600 nm with a spectrophotometer and a pH meter, respectively.

Structural Stability and Thermostability of AOBs. The structural stability of AOBs was examined by analyzing their surface properties (electrostatic repulsion and steric hindrance) that accounted for the aggregation of AOBs without fusion at a pH near their isoelectric points. In this examination, AOBs constituted with oleosin or caleosin were suspended in a medium of 0.1 M sodium phosphate buffer, pH 7.5, pH 6.5, or pH 4.5, and left at room temperature for 12 h before observation under a Nikon type 104 light microscope. To inspect the thermostability, AOBs were suspended in the sodium phosphate buffer,

left at various temperature conditions for 30 min, and then observed for their integrity under the light microscope.

RESULTS

Constitution of AOBs with Various Oil Body Proteins. In a previous study, it was demonstrated that artificial oil emulsions composed of TAG and PL without oil body proteins were unstable and fused to form large irregular oil drops (*13*). In this study, three oil body proteins, oleosin, caleosin, and steroleosin, were purified from sesame oil bodies (**Figure 1**). To detect if these three proteins could individually stabilize oil bodies, AOBs were constituted with each protein along with TAG and PL, and their stability was observed in a turbidity test (**Figure 2**). The results revealed that oleosin and caleosin, but not steroleosin, could encapsulate AOBs with a high stability similar to native oil bodies. As reported earlier (*18*), the sizes of AOBs constituted with oleosin were comparable to those of native oil bodies of 0.5−2 μm (**Figure 3**). In contrast, the sizes (approximately 50−200 nm in diameter) of AOBs constituted with caleosin were 10 times smaller than those constituted with oleosin.

AOBs Constituted with Native or Recombinant Caleosin. A sesame caleosin cDNA clone was transformed and overexpressed in *E. coli* to generate recombinant caleosin polypeptides (**Figure 4**). The success of overexpression was confirmed by western blotting; two bands were immunodetected presumably due to two different conformations of caleosin in association with or without calcium as reported earlier (*8*). Comparable sizes and stabilities were observed for the AOBs constituted with the recombinant caleosin in comparison with those constituted with native caleosin purified from sesame oil bodies (**Figure 2**). To compare the structural topology of native and recombinant caleosins in AOBs, both preparations were subjected to proteinase K digestion. A polypeptide of approximately 4 kDa, equivalent to the mass of the caleosin central hydrophobic domain, was observed in the proteolytic fragments of both AOBs after digestion (**Figure 5**). Presumably, both native and recombinant caleosins anchored to the surface of AOBs in a similar manner. In the following experiments, AOBs constituted with recombinant caleosin were used for further characterization.

Surface Properties and Stability of the AOBs Encapsulated by Caleosin. AOBs constituted with caleosin were subjected to isoelectrofocusing; the result indicated that their isoelectric point was around pH 4 (**Figure 6**). Therefore, the caleosin-stabilized AOBs were negatively charged and possessed electronegative repulsion to each other at neutral pH. Aggregation of these AOBs was induced by adjusting the pH of the suspension to 4.5 or lower (close to their isoelectric point) where the electronegative repulsion on their surface was nearly attenuated (**Figure 7**). Interestingly, the aggregated AOBs did not lead to coalescence and could be reversibly suspended as discrete particles when the suspension pH was adjusted back to a pH higher than 6.5 (data not shown). The thermostability of caleosin-stabilized AOBs was observed in a light microscope; they remained intact at temperatures lower than 50 °C, became partially unstable at 70 °C (perhaps due to some imperfectly encapsulated AOBs), and decomposed entirely at temperatures higher than 90 °C (**Figure 8**).

DISCUSSION

In this study, a novel type of AOB 10 times smaller than native seed oil bodies was assembled with TAG, PL, and caleosin. These caleosin-stabilized AOBs possessed a higher stability and were maintained as discrete particles at lower pH

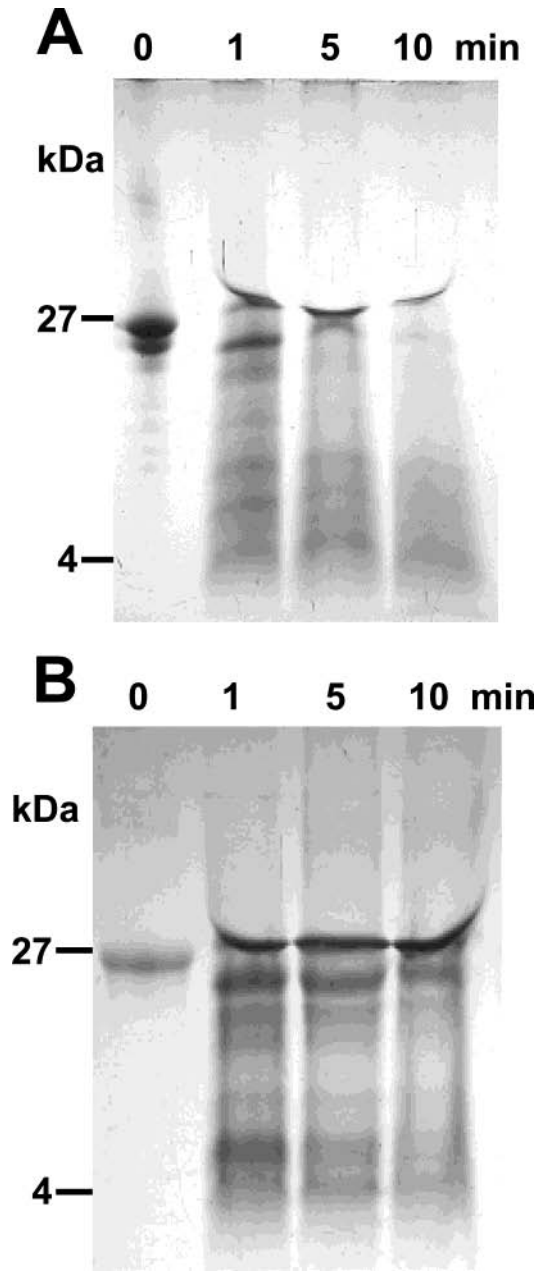

Figure 5. Protection of caleosin in AOBs from proteolytic digestion. AOBs constituted with native (**A**) or recombinant (**B**) calesoin were subjected to proteinase K digestion. The degraded fragments of caleosin at various intervals of digestion were resolved in SDS–PAGE with loading samples adjusted to represent amounts derived from an equal quantity of the reaction mixture. Labels on the left indicate the molecular masses of intact caleosin (27 kDa) and the leftover fragment (4 kDa).

surroundings. Their smaller size, superior structural integrity, higher thermostability, and better individuality in a wider pH range may render these artificially assembled particles a versatile vehicle for many potential applications.

To date, three oil body proteins, i.e., oleosin, caleosin, and steroleosin, have been identified in seed oil bodies. An oleosin molecule comprises three structural domains: an N-terminal amphipathic domain (4 kDa), a central hydrophobic oil body-anchoring domain (8 kDa), and a C-terminal amphipathic α-helical domain (4−6 kDa) (*11*). Similar to oleosin, caleosin also comprises three structural domains: an N-terminal hydrophilic calcium-binding domain (11 kDa), a central hydrophobic oil body-anchoring domain (4 kDa), and a C-terminal hydro-

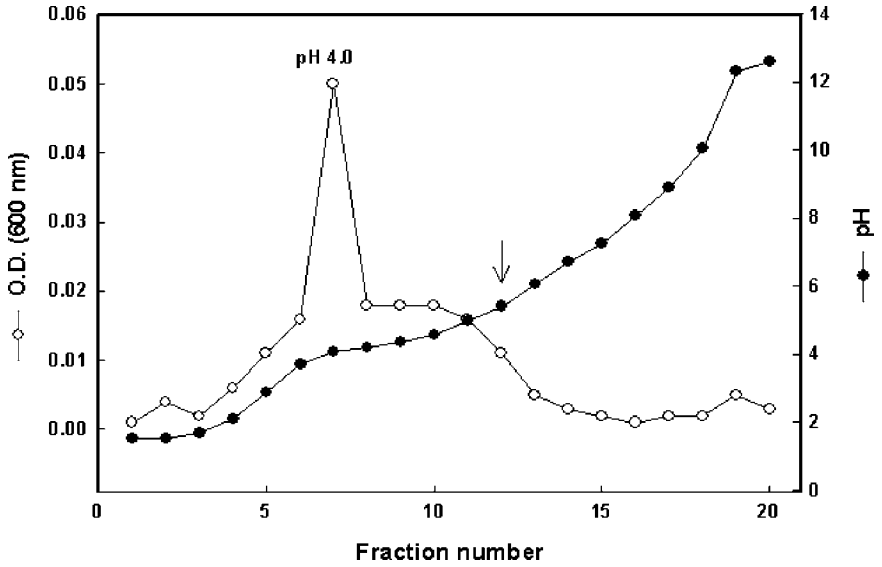

Figure 6. Isoelectrofocusing of AOBs constituted with caleosin. The pH gradient (close circles) was preformed before loading AOBs into the chamber. AOBs were loaded at the pH 6 region as indicated by an arrow. After they were electrofocused, the AOBs in the pH gradient were fractionated into 20 tubes. The distribution of the AOBs detected at OD 600 nm (open circles) was recorded for each tube.

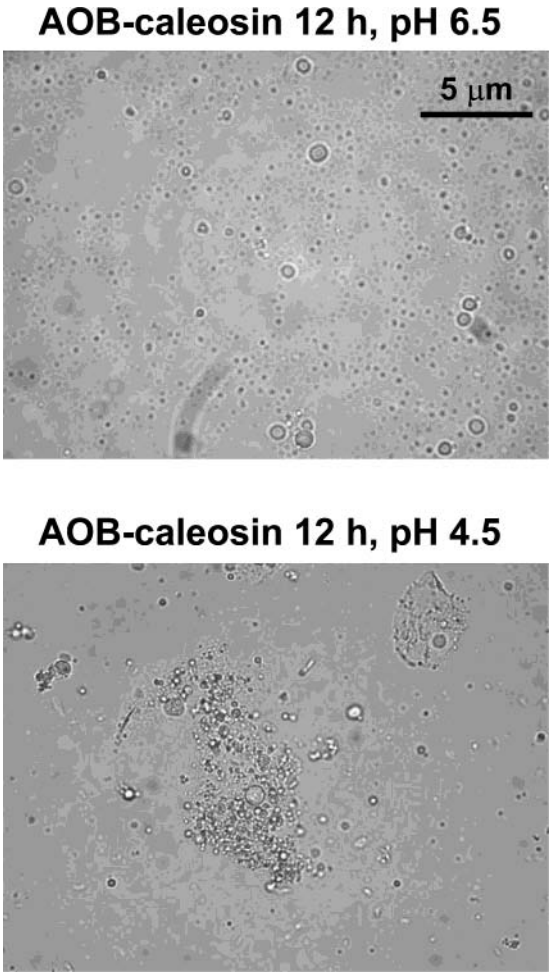

Figure 7. Light microscopy of AOBs at pH 6.5 and pH 4.5. The AOBs constituted with caleosin were kept at room temperature, pH 6.5 or pH 4.5, for 12 h before the photos were taken. Both photos are of the same magnification. The bar represents 5 μm.

philic phosphorylation domain (12 kDa) (*8*). A comparable motif termed proline knot is present in the middle of the central hydrophobic oil body-anchoring domains of both oleosin and caleosin and is presumably responsible for their targeting to the organelles (*20*). In contrast with oleosin and caleosin, steroleosin is proposed to comprise two structural domains: an N-terminal oil body-anchoring domain (4 kDa) and a soluble sterol-binding dehydrogenase domain (35 kDa) (*10*). This study showed that both oleosin and caleosin, but not steroleosin, could individually stabilize AOBs. It is likely that the structural organizations of oil body proteins are correlated to their capability of stabilizing oil bodies. The required topology for oil body stabilization seems to be a central hydrophobic domain flanked with protrusive domains on both sides.

The isoelectric points of native oil bodies purified from diverse seeds are pH 6.0−6.5 (*12*). AOBs constituted with oleosins as well as native oil bodies purified from sesame seeds are maintained as individual discrete particles at pH 7.5 but aggregate (without coalescence) at pH 6.5 (*18*, *22*). The aggregation of oil bodies at pH 6.5 is a result of the attenuation of electronegative repulsion among the organelles, and aggregated oil bodies do not coalesce due to steric hindrance of surface oleosins. In this study, with an isoelectric point of around pH 4, AOBs constituted with caleosin maintained as individual discrete particles at pH 6.5 but aggregated (without coalescence) at pH 4.5; obviously, the structural stability of these AOBs is also provided by electronegative repulsion and steric hindrance.

The sizes and surface properties of artificially assembled particles are crucial for their specific application (*23*, *24*). In our previous study, the sizes of AOBs constituted with oleosin could be controlled by the ratio of TAG/oleosin, and the stability of these AOBs was inversely correlated with their sizes (*18*). In comparison with native oil bodies, AOBs constituted with 10 times more oleosin, were 2−3 times smaller (average diameter of 0.85 μm), and possessed a higher thermostability up to 60 °C. In this study, AOBs constituted with caleosin were 10 times smaller with a higher thermostability up to 70 °C, as compared with those constituted with oleosin. The higher stability of AOBs constituted with caleosin presumably resulted from their smaller sizes and stronger electronegative repulsion on the surface. The sizes and properties of caleosin-stabilized AOBs provide an alternative source in considering artificially assembled particles that should fulfill the particular requirements for specialized applications.

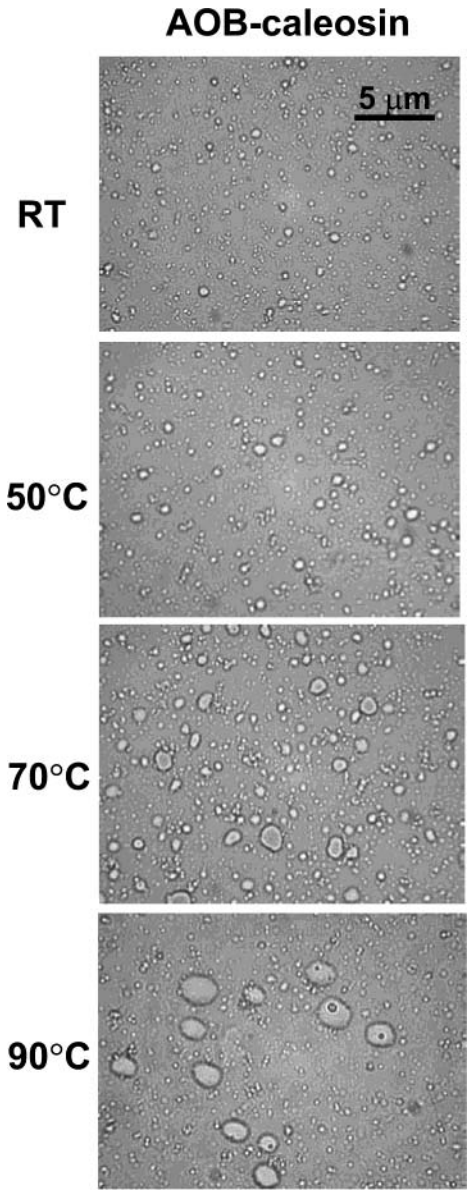

Figure 8. Light microscopy of AOBs under different temperature conditions. The AOBs constituted with caleosin were left at room temperature, 50, 70, and 90 °C for 30 min before the photos were taken. All photos are of the same magnification. The bar represents 5 μm.

ACKNOWLEDGMENT

We thank Professor Chih-Ning Sun for critical reading of the manuscript, Dr. Tien-Joung Yiu for supplying mature sesame seeds, and Ms. Pei-Chi Chao for the assistance in EM.

LITERATURE CITED

(1) Huang, A. H. C. Oleosin and oil bodies in seeds and other organs. *Plant Physiol.* **1996**, *110*, 1055−1061.
(2) Napier, J. A.; Stobart, A. K.; Shewry, P. R. The structure and biogenesis of plant oil bodies: the role of the ER membrane and the oleosin class of proteins. *Plant Mol. Biol.* **1996**, *31*, 945−956.
(3) Frandsen, G. I.; Mundy, J.; Tzen, J. T. C. Oil bodies and their associated proteins, oleosin and caleosin. *Physiol. Plant* **2001**, *112*, 301−307.
(4) Murphy, D. J. The biogenesis and functions of lipid bodies in animals, plants and microorganisms. *Prog. Lipid Res.* **2001**, *40*, 325−438.
(5) Tzen, J. T. C.; Huang, A. H. C. Surface structure and properties of plant seed oil bodies. *J. Cell Biol.* **1992**, *117*, 327−335.
(6) Tzen, J. T. C.; Peng, C. C.; Cheng, D. J.; Chen, E. C. F.; Chiu, J. M. H. A new method for seed oil body purification and examination of oil body integrity following germination. *J. Biochem.* **1997**, *121*, 762−768.
(7) Chen, E. C. F.; Tai, S. S. K.; Peng, C. C.; Tzen, J. T. C. Identification of three novel unique proteins in seed oil bodies of sesame. *Plant Cell Physiol.* **1998**, *39*, 935−941.
(8) Chen, J. C. F.; Tsai, C. C. Y.; Tzen, J. T. C. Cloning and secondary structure analysis of caleosin, a unique calcium-binding protein in oil bodies of plant seeds. *Plant Cell Physiol.* **1999**, *40*, 1079−1086.
(9) Næsted, H.; Frandsen, G. I.; Jauh, G. Y.; Hernandez-Pinzon, I.; Nielsen, H. B.; Murphy, D. J.; Rogers, J. C.; Mundy, J. Caleosins: Ca^{2+} binding proteins associated with lipid bodies. *Plant Mol. Biol.* **2000**, *44*, 463−476.
(10) Lin, L. J.; Tai, S. S. K.; Peng, C. C.; Tzen, J. T. C. Steroleosin, a sterol-binding dehydrogenase in seed oil bodies. *Plant Physiol.* **2002**, *128*, 1200−1211.
(11) Tzen, J. T. C.; Lie, G. C.; Huang, A. H. C. Characterization of the charged components and their topology on the surface of plant seed oil bodies. *J. Biol. Chem.* **1992**, *267*, 15626−15634.
(12) Tzen, J. T. C.; Cao, Y. Z.; Laurent, P.; Ratnayake, C.; Huang, A. H. C. Lipids, proteins, and structure of seed oil bodies from diverse species. *Plant Physiol.* **1993**, *101*, 267−276.
(13) Tzen, J. T. C.; Chuang, R. L. C.; Chen, J. C. F.; Wu, L. S. H. Coexistence of both oleosin isoforms on the surface of seed oil bodies and their individual stabilization to the organelles. *J. Biochem.* **1998**, *123*, 319−324.
(14) Tai, S. S. K.; Chen, M. C. M.; Peng, C. C.; Tzen, J. T. C. Gene family of oleosin isoforms in sesame seed oil bodies and their structural stabilization to reconstituted oil bodies. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2146−2153.
(15) Li, M.; Murphy, D. J.; Lee, K. H. K.; Wilson, R.; Smith, L. J.; Clark, D. C.; Sung, J. Y. Purification and structural characterization of the central hydrophobic domain of oleosin. *J. Biol. Chem.* **2002**, *277*, 37888−37895.
(16) Van Rooijen, G. J. H.; Moloney, M. M. Plant seed oil-bodies as carriers for foreign proteins. *Bio/Technology* **1995**, *13*, 72−77.
(17) Hou, R. C. W.; Lin, M. Y.; Wang, M. M. C.; Tzen, J. T. C. Increase of Viability of entrapped cells of *Lactobacillus delbrueckii ssp. bulgaricus* in artificial oil emulsion. *J. Diary Sci.* **2003**, *86*, 424−428.
(18) Peng, C. C.; Lin, I. P.; Lin, C. K.; Tzen, J. T. C.Size and stability of reconstituted sesame oil bodies. *Biotechnol. Prog.* **2003**, *19*, 1623−1626.
(19) Polson, A. Isolation of IgY from the yolks of eggs by a chloroform poly(ethylene glycol) procedure. *Immunol. Invest.* **1990**, *19*, 253−258.
(20) Chen, J. C. F.; Tzen, J. T. C. An in vitro system to examine the effective phospholipids and structural domain for protein targeting to seed oil bodies. *Plant Cell Physiol.* **2001**, *42*, 1245−1252.
(21) Chuang, R. L. C.; Chen, J. C. F.; Chu, J.; Tzen, J. T. C. Characterization of seed oil bodies and their surface oleosin isoforms from rice embryos. *J. Biochem.* **1996**, *120*, 74−81.
(22) Peng, C. C.; Tzen, J. T. C. Analysis of the three essential constituents of oil bodies in developing sesame seeds. *Plant Cell Physiol.* **1998**, *39*, 35−42.
(23) Davis, S. S. Biomedical applications of nanotechnology—implications for drug targeting and gene therapy. *Trends Biotechnol.* **1997**, *15*, 217−224.
(24) Davis, S. S.; Illum, L. Drug delivery. *Curr. Opin. Biotechnol.* **1991**, *2*, 254−259.

Received for review December 31, 2003. Revised manuscript received April 1, 2004. Accepted April 14, 2004. The work was supported by a grant from the National Science Council, Taiwan, ROC (NSC 91-2313-B-005-083 to J.T.C.T.).

JF035533G