Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 315 (2004) 224–227
BBRC
www.elsevier.com/locate/ybbrc

Biogenic capsules made of proteins and lipids

Gang Lu, Zhihua An, and Junbai Li*

International Joint Laboratory, Key Laboratory of Colloid and Interface Science, Institute of Chemistry, Chinese Academy of Sciences, Zhong Guan Cun, Bei Yi Jie No. 2, Beijing 100080, China

Received 11 January 2004

Abstract

Multilayer biogenic capsules with a micrometer scale were fabricated by self-assembly of proteins and lipids at the interface of emulsion droplets. The optical microscopy images demonstrate that spherical capsules at a fluid interface have uniform walls and the dried capsules possess a high mechanical strength. The hollow shells obtained provide a novel class of assembly with encapsulating drug molecules based on the layer-by-layer technique.

Keywords: Microcapsule; Protein; Lipid; Emulsion; Biocompatibility

Microencapsulation is an imperative technology in pharmacy and medicine. Great attention has been paid to develop encapsulation techniques for the purpose of controlled release and drug delivery [1]. Recently developed novel system like colloidosomes are permeable capsules composed of colloid particles [2,3]. Polyelectrolyte capsules, by coating size controlled templates with alternating polyelectrolyte adsorption and followed by removal of the templated cores, have been considered as a new type of nanoporous capsules [4–7]. For such a system, however, if core dimensions approach the persistence length and charge density for sufficient coating, this is especially demanding for biocompatible materials. For drug release and delivery in the medical and pharmaceutical areas it is realized that the coating materials of biocompatible capsules, prepared from biological molecules, would be of great advantage. Meanwhile, the encapsulation of drugs, proteins or enzymes requires a controlled way in physical structures, especially the compatibility and permeability.

Therefore, as an important extension of shell materials, the preparation of biogenic capsules with properties as good as hitherto for synthetic polymers is the next challenge. Proteins or their mixtures with lipids as efficient encapsulation materials can meet perfectly the demand of this application.

An adsorption layer of protein can obviously alter the properties of a surface. In emulsion systems, the addition of a low molecular weight surfactant like phospholipids interacting with the adsorbed protein layer at the interface serves as a stabilizer or an emulsifier [8–10]. Such a model system has been created by using a pendent drop technique, in which one drop of lipid chloroform solution was pumped precisely via a pipeline to the tip of a Teflon capillary and immersed into aqueous protein solution [11]. Co-adsorption of two components from immiscible solvents at the liquid/liquid interface reduces the competition of adsorption. In our previous work it has been demonstrated that such a complex can form a stable multilayer film [11,12].

Materials and methods

Human serum albumin (HSA) glycated (95%+) and L-α-Phosphatidic acid, dimyristoyl (approx 98%) (DMPA) were used as purchased from Sigma without further purification. Chloroform with 99% purity was obtained from Acros. All the water used in the experiments was three-stage Millipore grade, which has a resistivity higher than 18.2 MΩ cm.

HSA was dissolved in phosphate buffer solution at pH 3.8 with a concentration of 1.0 mg/mL. The net charge of HSA can be modified by pH around the isoelectric point (4.8). As much as 1.25 mg DMPA was dissolved in a mixed solvent (CH_3OH:$CHCl_3$ = 1:1, v/v) at a

* Corresponding author. Fax: +86-10-8261-2484.
E-mail address: jbli@iccas.ac.cn (J. Li).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.01.042

certain concentration and then the organic solvent was removed by evaporation in a rotavap at 35 °C. Afterwards DMPA was redispersed into pure water and diluted to the concentration of 0.025 mg/mL. Fluorescence microscopy images were obtained by Olympus IX 71.

Results and discussion

Here, we report on the fabrication of hollow protein/lipid capsules through self-assembly with sizes over submicrometer to micrometer scale and with typical biological and permeability features. By mixing dye molecules, rhodamine B, into the lipid/chloroform solution we have observed the dye distribution in the aqueous phase and demonstrated the penetration of the dyes through the walls of lipids/protein capsules. The fabrication process is very likely analogous to the emulsion process (Fig. 1). L-α-Phosphatidic acid, dimyristoyl (DMPA) chloroform solution is mixed with HSA buffer solution to form an immiscible fluid. A tiny solubility of chloroform in water could be diminished by a prior treatment [13]. By acute shaking, both HSA and DMPA (mixed with NBD labeled DPPE) adsorb on the surface of an emulsion droplet [11,12]. After the droplet surface is completely covered by the proteins and lipids, capsules exclusively are formed from biomaterials (Fig. 2). The microscopy image in Fig. 2A with fluorescently labeled walls shows well-defined capsules. The initially formed capsules possess a broad size distribution. The capsule size can be adjusted by centrifugation. The packing of HSA/DMPA complex films at the droplet surface affects the formation rate and stability of the capsules. The electrostatic interaction between DMPA and HSA may stabilize the interfacial layers and allow full coverage of the droplet [11,12]. A suitable ratio of the volume fraction of HSA to DMPA may lead to a complex film forming at the interface easily and tending to bend towards a spontaneous curvature. In addition, both concentrations of HSA and DMPA also determine the formation time and the stability of the capsules. The capsule size depends in some sense on the shaking. Too mild stirring will produce larger capsules. The adsorption of protein and lipids onto the interface of emulsion droplets is a typical self-assembly process and the assembled film can be very thick. This has been verified by a fully packed oil surface with polyelectrolyte [14–16].

Fig. 2B is a fluorescence micrograph of an air-dried hollow protein/lipid capsule that was produced by the self-assembly of DMPA and HSA. The drying process comprises an evaporation of the organic phase. It induces folds in the protein/lipid capsules. The dried protein/lipid capsule is transparent and possesses a high mechanical strength. The protein/lipid filled capsules maintain their original spherical shape. With fluorescence microscopy we have recorded the whole process of formation of the dried capsules with time as shown in Fig. 3. The capsules have been dyed with rhodamine B during shaping to allow the visualization by fluorescence

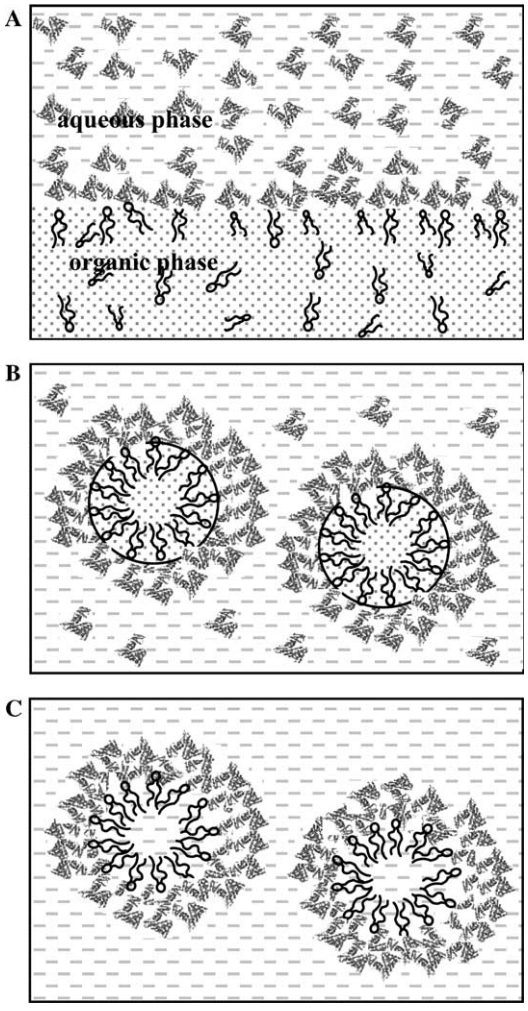

Fig. 1. Schematic illustration of the self-assembly process for HSA/DMPA microcapsules. (A) An immiscible interface is produced by HSA aqueous solution and DMPA chloroform solution. A complex film has been formed steadily by electrostatic and hydrophobic interaction. (B) HSA adsorbs onto the surface of DMPA/chloroform droplets via a gentle stirring. (C) After the droplet surface is completely covered by the complex film, a multilayer elastic shell is obtained through washing the coated droplets in buffer to remove the rest of the nonadsorbed materials.

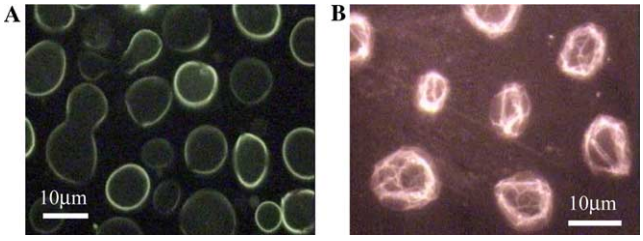

Fig. 2. (A) Optical image of tens of microcapsules composed of fluorescence labeled HSA and DMPA in an aqueous solution. HSA has been dyed with rhodamine B, allowing visualization with fluorescence microscopy. (B) The dried microcapsules made of HSA and DMPA.

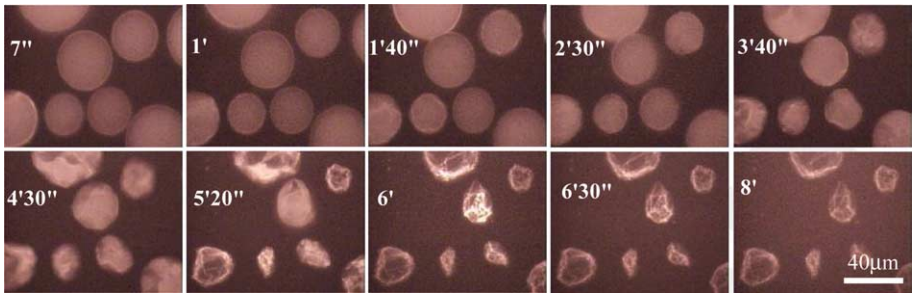

Fig. 3. Forming process of dried HSA/DMPA capsules obtained from solution. The capsules labeled with rhodamine B allowing visualization by fluorescence microscopy.

microscopy. The fully packed surfaces covered by the co-adsorption of proteins and lipids were formed on the chloroform droplets in aqueous solutions containing HSA. With increasing time the evaporated chloroform leads to droplet shrinking and forming a folded surface. Finally as the organic solvents were completely removed hollow capsules with an elastic shell were obtained.

The CD (A and B) and FTIR (C) spectrums of HSA aqueous solution and dried HSA microcapsules are shown in Fig. 4. The CD spectrum of HSA in a solution exhibits typically a shape for α-helix rich secondary structure (two minima at approximately 208 and 222 nm) (Fig. 4A) [17–19]. In the dried state of HSA, Fig. 4B shows no obvious variation of the α-helix

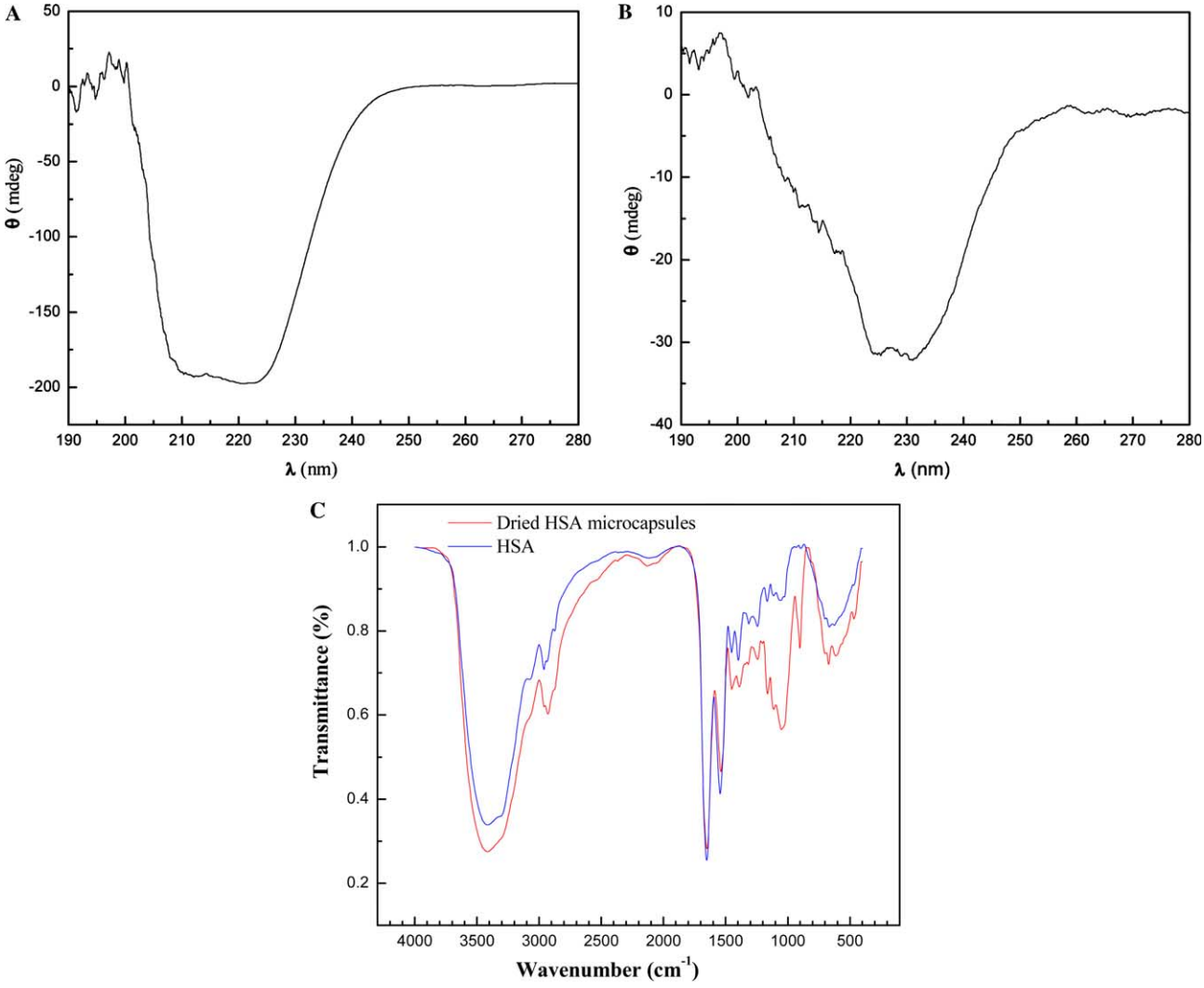

Fig. 4. (A) CD spectrum of HSA aqueous solution; (B) CD spectrum of the dried HSA microcapsules; and (C) FTIR spectra of HSA aqueous solution and the dried HSA microcapsules.

structure in corresponding to the two minima peaks. The IR spectrum of HSA in Fig. 4C also shows bands at 1652 and 1544 cm^{-1}, which can be assigned to amide I and amide II bands of α-helix rich secondary structure. These bands are observed in the dried HSA microcapsules as well, indicating they have the similar compositions.

The co-adsorption of proteins and lipids onto the surface of an emulsion droplet is a self-assembly process [11]. Multilayer biogenic capsules of HSA and DMPA with a micrometer scale can be fabricated with such an assembly. The optical microscopy images demonstrate that spherical capsules at a fluid interface have uniform walls and the dried capsules possess a high mechanical strength. The hollow shells obtained create a novel class of assembly system with encapsulation. The protein/lipid complex film provides highly stable and elastic shells that serve as a protecting coating for core materials [20]. Such HSA/DMPA capsules can be used potentially to encapsulate many species like particles, inorganic crystals or biological molecules with controllable permeability. The biomolecule pairing of HSA and DMPA matches many requirements for encapsulation, for instance, as drug carries. The obvious difference with the polymer capsules is their biological features, which are most likely biocompatible.

Acknowledgments

We gratefully acknowledge valuable discussions with Helmuth Möhwald. This work was financially supported by the National Nature Science Foundation of China (NNSFC29925307 and NNSFC90206035) as well as the collaborated project of German Max Planck Society.

References

[1] J.L. Brash, T.A. Horbett, Proteins at interfaces, in: T.A. Horbett, J.L. Brash, (Eds.), Proteins at Interfaces: Fundamentals and Applications, ACS Symposium Series 602, Washington, 1995, pp. 1–23.
[2] A.D. Dinsmore, M.F. Hsu, M.G. Nikolaides, M. Marquez, A.R. Bausch, D.A. Weitz, Colloidosomes: selectively permeable capsules composed of colloidal particles, Science 298 (2002) 1006–1009.
[3] Y. Lin, H. Skaff, T. Emrick, A.D. Dinsmore, T.P. Russell, Nanoparticle assembly and transport at liquid–liquid interfaces self-assembly, because the nanoparticle, Science 299 (2003) 226–229.
[4] G. Decher, Fuzzy nanoassemblies: toward layered polymeric multicomposites, Science 277 (1997) 1232–1237.
[5] F. Caruso, R.A. Caruso, H. Möhwald, Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating, Science 282 (1998) 1111–1114.
[6] E. Donath, G.B. Sukhorukov, F. Caruso, S.A. Davis, H. Möhwald, Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes, Angew. Chem. Int. Ed. 37 (1998) 2202–2205.
[7] Y. Lvov, Electrostatic layer-by-layer assembly of proteins and polyions, in: Y. Lvov, H. Möhwald (Eds.), Protein Architecture: Interfacing Molecular Assemblies and Immobilization Biotechnology, Marcel Dekker, New York, 2000, pp. 125–167.
[8] M. Bos, T. Nylander, T. Arnebrant, D.C. Clark, in: G.L. Hasenhuettl, R.W. Hartel (Eds.), Food Emulsifiers and Their Applications, Chapman and Hall, New York, 1997, pp. 95–146.
[9] M.J. Papiz, L. Sawyer, E.E. Eliopoulos, A.C.T. North, B.C. Findlay, R. Sivaprasadarao, T.A. Jones, J.P. Kraulis, The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein, Nature 324 (1986) 383–385.
[10] R.D. Fugate, P.S. Song, Spectroscopic characterization of β-lactoglobulin–retinaol complex, Biochim. Biophys. Acta 625 (1980) 28–42.
[11] J.B. Li, Y. Zhang, L.L. Yan, Multilayer formation on a curved drop, Angew. Chem. Int. Ed. 40 (2001) 891–894.
[12] G. Lu, H. Chen, J. Li, Forming process of folded drop surface covered by human serum albumin, β-lactoglobulin and β-casein, respectively, at the chloroform/water interface, Colloid Surf. A 215 (2003) 25–32.
[13] Taking into account the tiny solubility of chloroform in water the chloroform was saturated with Millipore water for all of the experiments.
[14] A. Voigt, H. Lichtenfeld, G.B. Sukhorukov, H. Zastrow, E. Donath, H. Bäumler, H. Möhwald, Membrane filtration for microencapsulation and microcapsules fabrication by layer-by-layer polyelectrolyte adsorption, Ind. Eng. Chem. Res. 38 (1999) 4037–4043.
[15] L. Dähne, S. Leporatti, E. Donath, H. Möhwald, Fabrication of micro reaction cages with tailored properties, J. Am. Chem. Soc. 123 (2001) 5431–5436.
[16] G.B. Sukhorukov, A.A. Antipov, A. Voigt, E. Donath, H. Möhwald, pH-controlled macromolecule encapsulation in and release from polyelectrolyte multilayer nanocapsules, Macromol. Rapid Commun. 22 (2001) 44–46.
[17] E.P. Vieira, H. Hermel, H. Möhwald, Change and stabilization of the amyloid-β(1–40) secondary structure by fluorocompounds, Biochem. Biophys. Acta 1645 (2003) 6–14.
[18] P. Wallimann, R.J. Kennedy, J.S. Miller, W. Shalongo, D.S. Kemp, Dual wavelength parametric test of two-state models for circular dichroism spectra of helical polypeptides: anomalous dichroic properties of alanine-rich peptides, J. Am. Chem. Soc. 125 (2003) 1203–1220.
[19] Y. Moriyama, K. Takeda, Re-formation of the helical structure of human serum albumin by the addition of small amounts of sodium dodecyl sulfate after the disruption of the structure by urea. A comparison with bovine serum albumin, Langmuir 15 (1999) 2003–2008.
[20] L.Q. Ge, H. Möhwald, J.B. Li, Stabilized phospholipid layers formed on polyelectrolyte multilayer capsules, Biochem. Biophys. Res. Commun. 303 (2003) 653–659.