Ultrasonics Sonochemistry 36 (2017) 454–465

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

A facile sonochemical synthesis of shell-stabilized reactive microbubbles using surface-thiolated bovine serum albumin with the Traut's reagent

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A R T I C L E I N F O

Article history:
Received 27 September 2016
Received in revised form 24 December 2016
Accepted 25 December 2016
Available online 26 December 2016

Keywords:
Microbubbles
BSA
Traut's reagent
Stability
Longevity
Adsorption

A B S T R A C T

The short lifetime of proteinaceous microbubbles produced using conventional sonication method has hindered their applications in drug delivery and metal removal from wastewater. In this study, we aimed to synthesize stable proteinaceous microbubbles and to demonstrate their reactivity. Our model protein, bovine serum albumin (BSA) was treated with 2-iminothiolane hydrochloride (Traut's reagent) to convert primary amines to thiols before the synthesis of microbubbles. Microbubbles produced with the Traut's reagent-treated BSA (BSA-SH MBs) were initially concentrated at median sizes of 0.5 and 2.5 μm. The 0.5 μm portion quickly vanished, and the 2.5 μm portion gradually shrank to ~850 nm in ~3 days and became stabilized afterward for several months under 4 °C. Characterizations of BSA-SH MBs by Fourier transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) indicated the presence of free unbound thiols and primary amines on their surface, implying the possibility of further surface modification. Based on the zeta potential measurement, the isoelectric point (IEP) of BSA-SH MBs was determined to be 4.5. The attachments of BSA-SH MBs on alumina, silica, and gold surfaces in different pH environments were carried out with a quartz crystal microbalance with dissipation monitoring (QCM-D), demonstrating the reactivities of BSA-SH MBs. At pH 6, the negatively charged BSA-SH MBs were adsorbed onto the alumina surface by electrostatic interaction. Analogously, at pH 4, the adsorption of the positively charged BSA-SH MBs on the silica surface was confirmed. Compared with the electrostatic interaction, the adsorption of BSA-SH MBs on the gold surface is attributed to the strong gold-thiol bonding effect. This is the first time that a universal approach for stabilizing protein-shelled microbubbles was reported using only one single step of surface treatment of proteins with the Traut's reagent.

1. Introduction

In the last decade, microbubbles have been utilized in clinical applications [1–11], food industries [12,13], and water treatment [14,15]. In clinical practice, as ultrasound contrast agents, microbubbles can improve the quality of the medical imaging [1–3,5–11]. Microbubbles have been employed as drug carriers [1–3,5,6] and the loading of therapeutic molecules onto or into the microbubbles can aid the coupling of imaging with therapeutics (theranostics) [7,8,11]. Microbubbles have also been used for surface attachments with other functional molecules such as magnetic, semiconductor nanoparticles, and stimulus-responsive polymers [4,9,10]. For example, Lentacker et al. developed polycation-coated microbubbles as potential gene delivery carriers [4]; Park et al. demonstrated ultrasound and magnetic resonance imaging applications of microbubbles loaded with metal, metal oxide or semiconductor nanoparticles [10]. Owing to their intrinsic antimicrobial properties, microbubbles have been intensively studied in the food industry as a sterilizing agent to depress the growth of *Bacillus* spores and *Escherichia coli* [12,13]. In wastewater treatment, ultrasonic radiation of microbubbles produces a vast amount of free radicals which can efficiently decompose organic compounds [14,15].

Without stabilizers, a short lifetime (~several hours) limits the use of conventional gas-filled microbubbles [16,17]. The pressure difference originating from the curved surface of the bubble, known as the Laplace pressure, spontaneously drives the entrapped gas to diffuse into the liquid phase [17,18]. Besides, Ostwald ripening adversely affects the lifetime of microbubbles; specifically, coalescence continues to reduce the surface energy of microbubbles until a complete phase separation is reached [19–21]. Surfactants can reduce the surface energy to lengthen the lifetime of microbubbles [22,23]; alternatively, biocompatible

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http://dx.doi.org/10.1016/j.ultsonch.2016.12.033
1350-4177/

polymers such as proteins and lipids can be employed to construct a rigid shell, which reduces outward gas diffusion [3,24,25]. Cavalieri et al. and Talu et al. respectively synthesized lysozyme-shelled and lipid-coated microbubbles with a lifetime of several months [24,25].

The formation of disulfide bond has shown to increase the stability of microbubbles [25–28]. During the process of acoustic cavitation, superoxide is generated, cross-linking free thiols of cysteine residues [25]. A higher disulfide content contributes to a thicker shell and thus to a greater stability of microbubbles [27]. Free thiols can be released internally from proteins with reducing agents such as DL-dithiothreitol (DTT) to enhance inter- and intramolecular disulfide bridging during microbubble formation [25,26,28]. However, such treatment might excessively loosen up the protein structure and gives rise to a lowered hydrophobicity, which reduces the stability of microbubbles [27].

In this paper, we reported, for the first time, a facile synthesis of long-lived microbubbles with surface-treated proteins. Traut's reagent, which reacts with primary amines (Fig. 1a), was applied to our model protein BSA to render thiol groups externally [29]. Upon the ultrasonic radiation, the thiolated BSA (BSA-SH) forms the shell of microbubbles with air entrapped inside (BSA-SH MBs). In approximately 3 days, BSA-SH MBs gradually shrank to a critical size of ~850 nm while forming a thicker shell. As a result of bubble shrinkage, the increased Laplace pressure was balanced by the thickened shell, reaching an equilibrium and stabilizing for several months. The characterizations of microbubbles by Fourier transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) indicated the presence of primary amines and thiols after the microbubble synthesis. Quartz crystal microbalance with dissipation monitoring (QCM-D) illustrated their reactivities on silica, alumina, and gold surfaces, shedding light on the possibil-

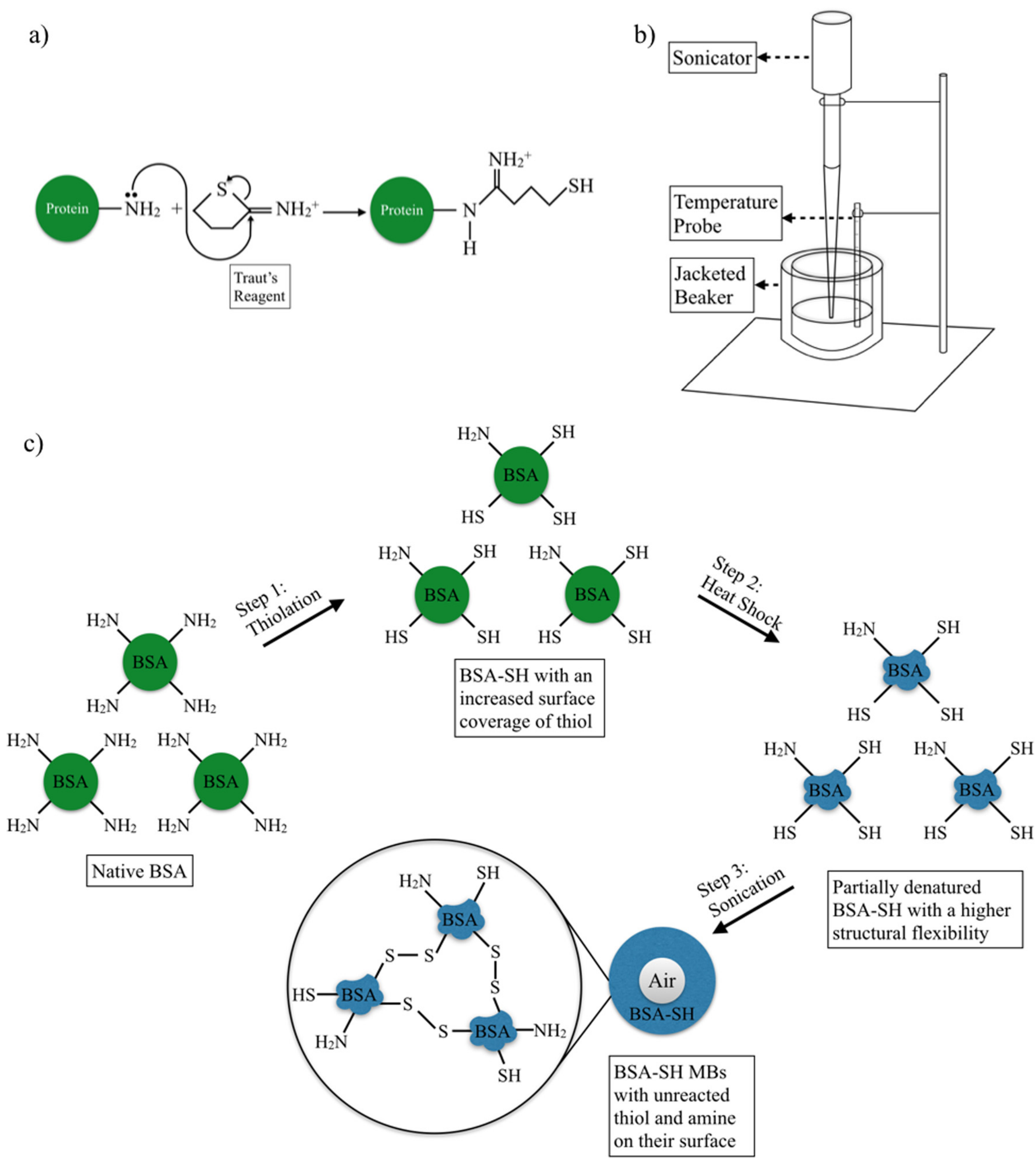

Fig. 1. Schematics for the mechanism of thiolation of a protein with the Traut's reagent (a), experimental setup of sonication (b), and flow charts of synthesis os BSA-SH MBs (c).