Available online at www.sciencedirect.com
ScienceDirect
Ultrasonics Sonochemistry 14 (2007) 173–183
Ultrasonics SONOCHEMISTRY
www.elsevier.com/locate/ultsonch

Structural and functional changes in ultrasonicated bovine serum albumin solutions

İbrahim Gülseren [a], Demet Güzey [b], Barry D. Bruce [c], Jochen Weiss [b,*]

[a] Department of Food Science, Pennsylvania State University, 126 Borland Laboratory, University Park, PA 16802, USA
[b] Food Biophysics and Nanotechnology Laboratories, Department of Food Science, University of Massachusetts, 234 Chenoweth Lab, 100 Holdsworth Way, Amherst, MA 01003, USA
[c] Department of Biochemistry, Cellular and Molecular Biology Walters Life Science Building, The University of Tennessee, 1414 W. Cumberland Avenue, Knoxville, TN 37996, USA

Received 1 January 2005; received in revised form 1 June 2005; accepted 1 July 2005
Available online 7 September 2006

Abstract

Effects of high-intensity ultrasonication on functional and structural properties of aqueous bovine serum albumin (BSA) solutions were investigated. The functional properties of BSA were altered by ultrasonication. Surface activity of BSA increased. Minimal changes were observed in the global structure of BSA but surface charge increased particularly at basic pH values (e.g. pH > 9). While dynamic light scattering measurements indicated that the particle size increased up to 3.4 times after 90 min of sonication, no significant increase in the oligomeric state of BSA using blue native PAGE was observed. The amount of free sulfhydryl groups in BSA after 90 min of sonication decreased. The increased particle size and decreased number of free sylfhydryl groups may be attributed to formation of protein aggregates. Surface hydrophobicity increased and circular dichroism spectroscopy and FTIR analysis indicated changes in the secondary structure of BSA. We hypothesize that mechanical, thermal and chemical effects of ultrasonication resulted in structural changes in BSA that altered the functional properties of the macromolecule which may be attributed to the formation of an ultrasonically induced state that differs from a thermally, mechanically or solvent induced state.

Keywords: High-intensity ultrasound; Sonication; BSA; Structure; Functionality; Modification

1. Introduction

An emerging processing technology increasingly used in chemical synthesis, preparation of pharmaceuticals and food processing is high-intensity ultrasonication. High-intensity ultrasonication is based on the interaction of sound waves with frequencies ranging from 16 kHz to several hundred kHz with biological or synthetic materials [1]. In contrast to non-destructive, diagnostic ultrasound with frequencies in the MHz range; application of high-intensity ultrasonic waves may alter material properties. The thermal, mechanical and chemical effects of high-intensity ultrasound have been attributed to the rapid formation and collapse of cavitational bubbles generating intense normal and shear stresses [2,3]. These bubbles collapse in the positive pressure cycle and produce highly turbulent flow conditions and extreme pressures and temperatures. Transient temperatures of up to 5000 K and pressures up to 1200 bar have been calculated [4]. Activity of acoustic waves has been reported to initiate heterogeneous reactions in the absence of interfering solvents [5]. Cavitational thermolysis may produce hydroxyl radicals and hydrogen atoms that can be followed by formation of hydrogen peroxide and, in the absence of oxygen, hydroperoxyl radicals [6–8]. Consequently, high-intensity ultrasound has been used to initiate and accelerate radical-driven reactions such as ring-open polymerization, modify system morphologies that develop for example during crystallization, and accelerate mass

* Corresponding author. Tel.: +1 413 545 1025; fax: +1 413 545 1262.
E-mail address: jweiss1@foodsci.umass.edu (J. Weiss).

1350-4177/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ultsonch.2005.07.006

transport processes that are of importance in mixing, extraction and drying [9,1,10–12].

Increasingly, the application of high-intensity ultrasound to modify biopolymers is being studied. For example, hydrolysis and cleavage has been reported for a variety of polysaccharides [5]. Sonication of starch led to formation of shorter chain molecules and reducing sugars [13]. Partially depolymerized dextrans, of great interest to the pharmaceutical industry, were produced via sonochemical degradation of native dextrans [14]. The homolytical/heterolytical midchain splitting of dextran together with a recombination of the fragments led to a narrower mass distribution.

The effect of high-intensity ultrasound on the structural and functional properties of proteins has been less studied. Generally, the functional properties of proteins (e.g. organoleptic, kinesthetic, hydration, interfacial, enzymatic and rheological properties) which are of key interest to manufacturers of pharmaceutical, food and cosmetic products, are influenced by their molecular structure that determines inter- and intra-molecular interactions e.g. formation of covalent and/or non-covalent bonds [11]. The structure–function relationship in proteins may be altered during processing due to the initiation of chemical and enzymatic reactions that include oxidation, glycosylation, hydroxylation, phosphorylation, methylation and acylation. These reactions depend on increases or decreases in process temperature and the application of mechanical forces such as high pressure or shear [15–17]. Because of the mechanism of action of high-intensity ultrasound, one would expect alterations in both structure and function of proteins upon application of high-intensity ultrasound but reported results on alterations of enzymatic activity varied [18–21]. Özbek and Ülgen reported a 70% inactivation of glucose-6-phosphate dehydrogenase with inactivation being affected by the sonication time while alkaline phosphatase under the same circumstances remained fully active [21]. Barton and coauthors reported an increase in invertase activity in sugar hydrolysis upon ultrasonication, which was explained by improvements in mixing [18]. Vargas recently reported similar results for invertase activity from *Aspergillus niger* and argued that enzyme activity was not altered but that the enzyme was released in higher quantities from cells which resulted in an acceleration of sugar hydrolysis [19].

The objective of this study was to investigate selected structural and functional properties in a well-defined model protein, bovine serum albumin (BSA), upon ultrasonication to obtain a better understanding of the physicochemical effects of ultrasound on this important class of biological macromolecules.

2. Materials and methods

2.1. Materials

2.1.1. Protein

Bovine serum albumin (Fraction V, min 98%) was purchased from Sigma Chemicals. BSA contains 35 half-cystines (i.e. 17 disulfide bridges and 1 free sulfhydryl group at residue 34), has a globular tertiary structure and its secondary structure consists of mainly α-helices [22]. The protein had been fractionated by heat shock, de-lipidized and dialyzed but was not completely fatty acid free.

2.1.2. Solvents

10 × PBS (phosphate buffered saline) solution was purchased from BioWhittaker. HPLC grade water was obtained from Fluka. Sodium hydroxide and deuterium oxide (99.9 atom% D for Fourier-transform infrared spectroscopy) was purchased from Sigma Chemical Company. Hydrochloric acid was obtained from Fisher Scientific.

2.1.3. Reactants and probes

Fluorescent probes 1-(anilino)-naphtalene-8-sulphonate (ANS^-) and 6-propionyl-2-(*N*,*N*-dimethyl-amino) naphthalene (PRODAN) probes were obtained from Molecular Probes. For Ellman's assay, DTNB [5,5′-dithio-bis(2-nitrobenzoic acid)], anhydrous mono- and dibasic sodium phosphate (>99% purity) were purchased from Sigma Chemicals. L-Cysteine hydrochloride monohydrate, potassium chloride (min 99% purity), acrylamide 40% stock (acyrlamide:bis-acrylamide = 37.5:1), bis-acrylamide, bistris/HCl, TEMED (*N*,*N*,*N*′,*N*′-Tetramethylethylenediamine) and tricine were purchased from Fisher Scientific. Coomassie brilliant blue G-250 and APS were obtained from Sigma Chemical Company. Aminocaproic acid and glycerol were purchased from Avocado Research Chemicals and JT Baker, respectively.

2.2. Methods

2.2.1. Solution preparation

Unless otherwise stated, 3×10^{-4} M BSA solutions were prepared in 0.1 × PBS solutions. Concentrations were verified by measuring the adsorbance at 279 nm ($\epsilon_{BSA,1\%,1cm} = 6.67$) using a UV–visible spectrometer (HP 5801A, Palo Alto, CA). Solution pH of 7.4 was determined with a pH Meter (MP220, Mettler-Toledo). To remove additional impurities, stock protein solutions were passed through a filter with 0.22 μm pore width (Millipore Corporation). All BSA solutions were prepared at room temperature at least 2 h prior to use and were stirred thoroughly to ensure proper hydration of proteins. Solutions were stored at 4 °C in a refrigerator and used within a day. All solutions were prepared with distilled and deionized water (Milli-Q, Millipore Corporation) and sodium azide (0.02%) was added as an antimicrobial agent.

2.2.2. High-intensity ultrasound treatment

An ultrasonic processor (Model 550, Misonix Incorporated, Farmingdale, NY) with a stainless steel probe with a diameter of $\frac{1}{2}$ in. was used to sonicate 30 ml of protein solutions in 50 ml centrifuge tubes that were immersed in a temperature-controlled (2 °C) water bath (Lauda RM6, Germany). The solutions were treated at a power setting of 7 up to 90 min. The ultrasonic intensity of the generated