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Al cation induces aggregation of serum proteins

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ABSTRACT

Al cation is known to induce protein fibrillation and causes several neurodegenerative disorders. We report the spectroscopic, thermodynamic analysis and AFM imaging for the Al cation binding process with human serum albumin (HSA), bovine serum albumin (BSA) and milk beta-lactoglobulin (b-LG) in aqueous solution at physiological pH. Hydrophobicity played a major role in Al–protein interactions with more hydrophobic b-LG forming stronger Al–protein complexes. Thermodynamic parameters ΔS, ΔH and ΔG showed Al–protein bindings occur *via* hydrophobic and H-bonding contacts for b-LG, while van der Waals and H-bonding interactions prevail in HSA and BSA adducts. AFM clearly indicated that aluminum cations are able to force BSA and b-LG into larger or more robust aggregates than HSA, with HSA 4 ± 0.2 (SE, $n = 801$) proteins per aggregate, for BSA 17 ± 2 (SE, $n = 148$), and for b-LG 12 ± 3 (SE, $n = 151$). Thioflavin T test showed no major protein fibrillation in the presence of Al cation. Al complexation induced major alterations of protein conformations with the order of perturbations b-LG > BSA > HSA.

1. Introduction

Aluminum is found naturally in the environment and you are always exposed to some aluminum by eating food, drinking water, ingesting medicinal products like certain antacids that contain aluminum, or breathing air [1]. Until today, no biological function has been assigned to this metal. On the contrary, Al accumulation in tissues and organs results in their dysfunction and toxicity, effects that usually correlate with the local concentration of the Al cation [2,3]. Although not fully proven, Al accumulation in the brain is proposed to be associated with neurodegenerative diseases, including Alzheimer's dementia, Parkinson's disease [4]. It has been shown that metal ions including Al cation may trigger protein structural changes such as aggregation and fibrillation [5,6]. In addition, the relationship between Al cation and the pathogenesis of Alzheimer's disease has been well investigated [7–10]. The aim of this study was to determine the effect of Al cations on the structural transformations of serum proteins.

To investigate the effect of Al cation complexation on protein structure in solution, the bindings of Al to human serum albumin, bovine serum albumin and beta-lactoglobulin were investigated. HSA and BSA have similar folding, well known primary structures [11,12] and are considered as models for studying drug–protein interaction *in vitro*. Even though there are marked structural similarities between HSA and BSA some differences are observed in the hydrophobicity of these two proteins [13]. Similarly, b-LG shows more hydrophobicity than HSA and BSA [14,15]. Such differences in hydrophobicity lead to a different affinity of HSA, BSA and b-LG toward Al complex formation. Even though complexation of Al with proteins are known [16–20], thermodynamic and microscopic analysis of Al–protein adducts are not fully investigated. In view of the differences in the hydrophobicity of HSA, BSA and b-LG it was of interest to examine the interaction of Al cation with these model proteins and the effect of cation binding on protein aggregation and fibrillation.

We now report, thermodynamic parameters, spectroscopic analysis and AFM imaging for the complexation of Al with HSA, BSA and b-LG in aqueous solution. The Al cation binding modes and the protein aggregation and fibrillation are discussed here.

2. Experimental

2.1. Materials

HSA, BSA fraction V and β-lactoglobulin (A variant, purity >90) were obtained from Sigma Chemical Company (St-Louis, MO) and used as supplied. Hydrated $AlCl_3$ was from Aldrich Chemical Co and

Abbreviations: HSA, human serum albumin; BSA, bovine serum albumin; b-LG, beta-lactoglobulin; FTIR, Fourier transform infrared; AFM, atomic force microscopy.

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used as supplied. Other chemicals were of reagent grade and used without further purification.

2.2. Preparation of stock solutions

Protein BSA or HSA (10 mg/ml) or b-LG (3 mg/ml) was dissolved in aqueous solution (120 μM) containing 10 mM Tris–HCl buffer (pH 7.4). The serum protein concentration was determined spectrophotometrically using the extinction coefficient 35,219 M^{-1} cm^{-1} at 280 nm [21]. The beta-lactoglobulin content was estimated, using the extinction coefficients of 17,600 M^{-1} cm^{-1} (MW = 18 kD) at 280 nm [22]. Hydrated $AlCl_3$ was dissolved in 10 mM of Tris–HCl solution (pH 7.4) and diluted to various concentrations.

2.3. AFM methodology

1:1 mixtures of aluminum chloride and HSA, BSA and b-LG at a concentration of 120 μM in 10 mM Tris–HCl, pH 7.4 were diluted 100 times in nanopure water. Freshly cleaved mica disks around 1 cm in diameter were incubated with 30 μml of sample solution for 1 min and washed thoroughly with several milliters of nanopure water. The mica disks were then dried with compressed nitrogen and imaged in AC mode with an Agilent 5500 atomic force microscope (Keysight, USA). We used NTESP silicon cantilevers (Bruker, USA) with a typical resonant frequency around 300 kHz and a nominal tip radius of 10 nm. Images were acquired at a scan rate of 1 Hz and analyzed using the free software Gwyddion. For the measurement of particle sizes, the particles were marked using a height threshold of 0.5 nm and the pixel size was 1 nm for all the images used. In order to remove false-positive particles we only present results for particles with an equivalent radius above 3 nm.

To estimate the number of proteins per aggregate based on their dry volume, we used the molecular weight HSA, BSA and b-LG to estimate their hydrated volume assuming a protein density of 1.37 g/cm^3. Assuming all the water leaves the protein upon drying which is an over simplification, the dry volume should be 37% of the wet volume, which gives us 30 nm^3 for HSA and BSA, and 8.2 nm^3 for b-LG.

2.4. UV–visible spectroscopy

The UV–vis spectra were recorded on a Perkin-Elmer Lambda spectrophotometer with a slit of 2 nm and scan speed of 400 nm min^{-1}. Quartz cuvettes of 1 cm were used. The absorbance measurements were performed at pH 7.4 by keeping the concentration of protein constant (60 μM), while increasing Al cation concentrations (1–60 μM).

The binding constants of Al–protein adducts were obtained according to the method described as reported [23,24].

2.5. FTIR spectroscopic measurements

Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model), equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter, using AgBr windows. Solution of Al cation was added dropwise to the protein solution with constant stirring to ensure the formation of homogeneous solution and to reach the target Al concentrations of 15, 30 and 60 μM with a final protein concentration of 60 μM. Spectra were collected after 2 h incubation of HSA, BSA and b-LG with Al solution at room temperature, using hydrated films. Interferograms were accumulated over the spectral range 4000–600 cm^{-1} with a nominal resolution of 2 cm^{-1} and 100 scans. The difference spectra [protein solution + Al solution – (protein solution)] were generated using water combination mode around 2300 cm^{-1}, as standard [25]. When producing difference spectra, this band was adjusted to the baseline level, in order to normalize difference spectra.

Analysis of the secondary structures of HSA, BSA and b-LG and their Al complexes were carried out on the basis of the procedure previously reported [26,27]. The curve-fitting analysis was performed using the GRAMS/AI Version 7.01 software of the Galactic Industries Corporation.

2.6. Fluorescence spectroscopy

Fluorimetric experiments were carried out on a Varian Cary Eclipse. Solution containing thioflavin T 25 μM in Tris–HCl (pH = 7.4) was prepared at room temperature and maintained at 24 °C. Solutions of HSA, BSA and b-LG containing 50 μM in 10 mM Tris–HCl (pH = 7.4) were also prepared at 24 °C. The fluorescence spectra were recorded at excitation 450 nm and emission from 480 to 20 nm.

3. Results and discussion

3.1. AFM analysis and ultrastructures of Al–protein adducts

For the three serum proteins analyzed here, we observed the presence of aggregates on the mica surface (Fig. 1). A protein concentration of 1.2 μM was used for incubation to limit unwanted crowding of the aggregates on the surface. As a result we were able to measure the average volume of the protein aggregates and estimate the number of protein per aggregates assuming that all the water is removed upon drying. For HSA we obtained 4 ± 0.2 (SE, n = 801) proteins per aggregate, for BSA 17 ± 2 (SE, n = 148), and

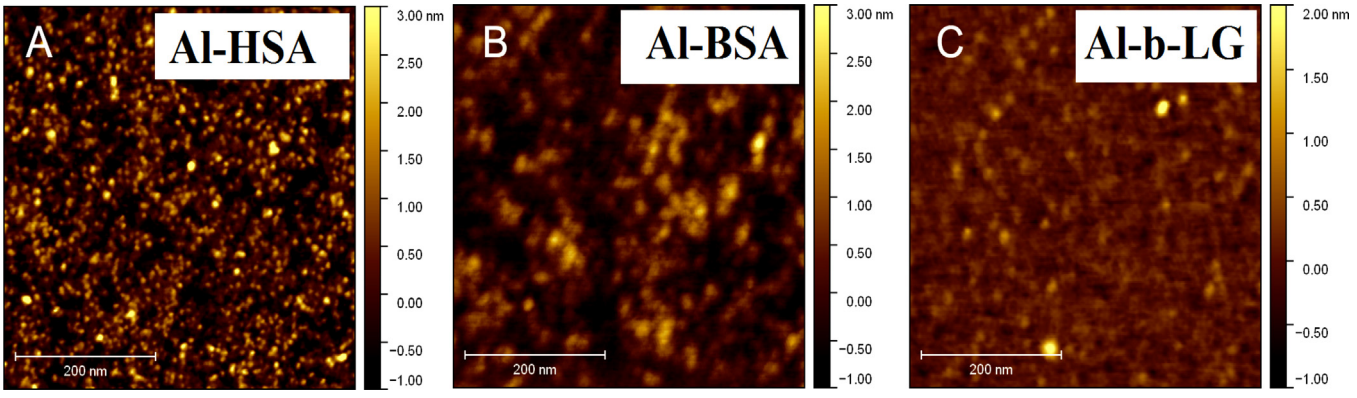

Fig. 1. AFM height images of the three serum protein aggregates induced by aluminum cations, (A) HSA, (B) BSA, (C) b-LG. Notice the presence of elongated aggregates for the BSA sample.