Colloids and Surfaces B: Biointerfaces 133 (2015) 263–269

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Fluorescence behavior of globular proteins from their bulk and thin film conformations in presence of mono-, di- and tri-valent ions

Ashim Chandra Bhowal, Kaushik Das, Sarathi Kundu*

Physical Sciences Division, Institute of Advanced Study in Science and Technology, Vigyan Path, Paschim Boragaon, Garchuk, Guwahati, Assam 781035, India

ARTICLE INFO

Article history:
Received 17 February 2015
Received in revised form 1 June 2015
Accepted 3 June 2015
Available online 12 June 2015

Keywords:
BSA
Lysozyme
Protein films
Photoluminescence
UV–vis
AFM

ABSTRACT

Photoluminescence behavior of globular proteins, lysozyme and bovine serum albumin (BSA), from their bulk and thin film conformations have been studied in presence of mono-, di- and tri-valent ions by using fluorescence and UV-Vis spectroscopy at two different temperatures and the morphology of the protein thin films have been studied by using atomic force microscopy. Protein- and ion-dependent dynamic and static quenching behaviors have been identified. While dynamic quenching is observed for lysozyme for all the three different valent ions, BSA shows no quenching for mono-valent (Na^+) ions, dynamic quenching for di-valent (Ni^{2+}) ions and static quenching for tri-valent (Fe^{3+}) ions at pH ≈ 5.5. After heat treatment, as the conformation of the protein molecules changes, the quenching efficiency for lysozyme in presence of ions decreases but shows enhancement for BSA. In thin film geometry, the molecular conformation of both lysozyme and BSA modifies on the solid surfaces and hence quenching efficiency also modifies in comparison with that of bulk and as a result the quenching efficiency for lysozyme increases but decreases for the BSA film.

1. Introduction

Proteins are unique biopolymers which can display useful intrinsic fluorescence [1]. The three aromatic amino acids, i.e., phenylalanine, tyrosine and tryptophan, which are present in the proteins can absorb and emit light. Among the three aromatic amino acids tryptophan shows most intense fluorescence [1,2]. However, the fluorescence of tryptophan is highly sensitive to its local environment [1,3,4]. Conformational change, substrate binding, solvent polarity, ionic environment, etc. can disturb the microenvironment around the tryptophan residues and thus influences on the fluorescence emission. Fluorescence quenching of tryptophan occurs as the excited state indole donate electrons to the specific absorbers, like molecules, ions, etc. [5–7]. Tryptophan can be quenched by the addition of external quenchers or by nearby groups within the proteins [8–15]. Proteins possess a very complex three-dimensional structure with various conformational levels of folding [16,17]. Variation of different physicochemical conditions such as protein concentration, solution pH, temperature, ionic strength, substrate binding, etc. can make the protein unfold which results the conformational changes in the protein structure and hence influences on the tryptophan emission behaviors. Tryptophan fluorescence is thus indirectly used to monitor the protein conformational changes [18].

Globular proteins bovine serum albumin (BSA) and lysozyme are very fascinating model proteins due to their chemical, physical and biomedical functions [19–22]. Biochemical and biophysical function of proteins varies when their structure modifies. Both BSA and lysozyme molecules contain two and six tryptophan residues, respectively. Depending upon the protein, tryptophan can stay on the protein surface and/or inside the protein molecule [23–27]. While the surface tryptophan residues are easily accessible to the solvent and dissolved ions, tryptophan resides which are located inside the hydrophobic core has less accessibility to the solvent and ions. Depending upon their interaction behavior with the molecules or ions, the emission behavior modifies. Although different studies are going on, but the effect of ions on the emission behaviors of proteins below and above their isoelectric point and at their bulk and thin film conformations have not yet been explored properly.

In this article, we have studied the absorption and emission behaviors of lysozyme and BSA proteins in the presence of mono-valent (Na^+), di-valent (Ni^{2+}) and tri-valent (Fe^{3+}) ions at pH ≈ 5.5 by using fluorescence and UV-Vis spectroscopy. In addition, thin films of lysozyme and BSA on silicon substrates have been prepared and their morphological and emission behaviors have been studied using atomic force microscopy and fluorescence spectroscopy, respectively. Dynamic quenching is effectively observed for lysozyme for all the three different valent ions at pH ≈ 5.5.

* Corresponding author. Tel.: +91 361 2912073; fax: +91 361 2273062.
E-mail address: sarathi.kundu@gmail.com (S. Kundu).

http://dx.doi.org/10.1016/j.colsurfb.2015.06.010
0927-7765/

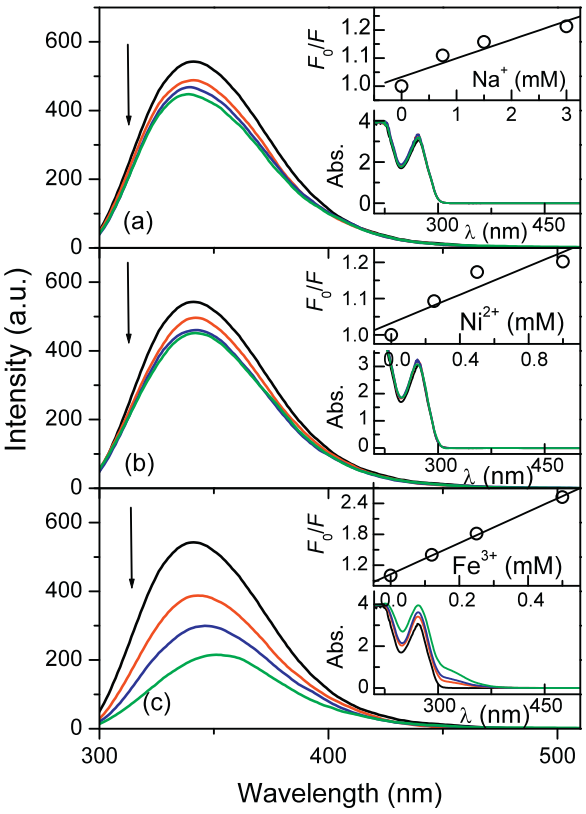

Fig. 1. Fluorescence emission spectra of lysozyme solutions in presence of ions. (a) Mono-valent (NaCl) salt: 0.0, 0.75, 1.50 and 3.00 mM, (b) di-valent ($NiCl_2$) salt: 0.0, 0.25, 0.50 and 1.00 mM, and (c) tri-valent ($FeCl_3$) salt: 0.0, 0.12, 0.25 and 0.50 mM. Arrows indicate the direction of the increasing salt concentrations. Lower insets: corresponding UV–vis absorbance spectra. Upper insets: corresponding Stern–Volmer plot of lysozyme with increasing salt concentrations.

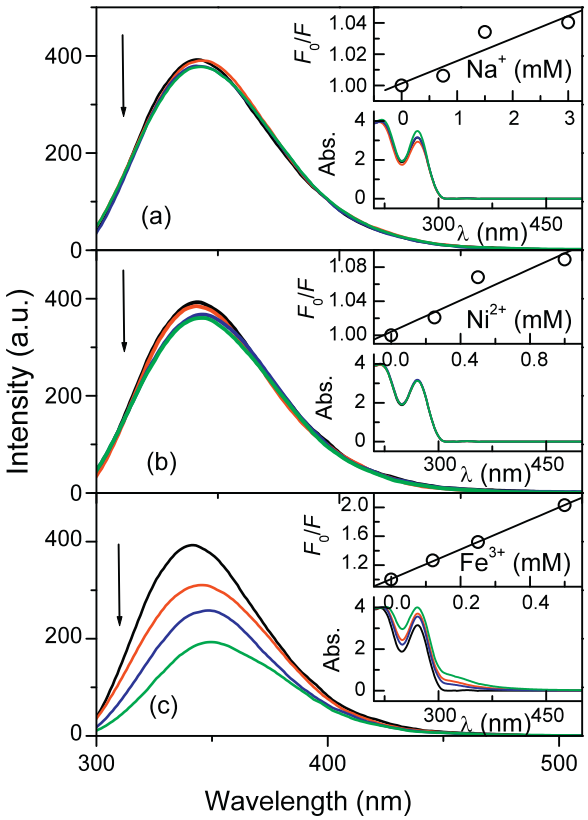

Fig. 2. Fluorescence emission spectra of lysozyme solutions after heat treatment in presence of ions. (a) Mono-valent (NaCl) salt: 0.0, 0.75, 1.50 and 3.00 mM, (b) di-valent ($NiCl_2$) salt: 0.0, 0.25, 0.50 and 1.00 mM, and (c) tri-valent ($FeCl_3$) salt: 0.0, 0.12, 0.25 and 0.50 mM. Arrows indicate the direction of the increasing salt concentrations. Lower insets: corresponding UV–vis absorbance spectra. Upper insets: corresponding Stern–Volmer plot of lysozyme with increasing salt concentrations.

On the other hand, BSA shows no quenching for mono-valent (Na^+) ions, dynamic quenching for di-valent (Ni^{2+}) ions and static quenching for tri-valent (Fe^{3+}) ions at pH ≈ 5.5. After the heat treatment, as the conformation of the protein molecule changes, the quenching efficiency for lysozyme in presence of ions decreases but enhances for BSA. Moreover, for the lysozyme and BSA thin films, the quenching efficiency modifies in comparison with their bulk values due to their conformational changes. Quenching efficiency for the lysozyme film increases but decreases for the film of BSA.

2. Experimental

Bovine Serum Albumin (BSA) (catalog No. A2153) and lysozyme (catalog No. 62971) were purchased from Sigma. 0.25 wt% BSA and 0.125% lysozyme were taken for all the measurements as final solution to do the absorbance and fluorescence measurements. The effect of ions on proteins is studied by dissolving monovalent (NaCl), divalent ($NiCl_2$) and trivalent ($FeCl_3$) salts in the protein solutions. Salt concentrations were varied from 0.25 mM to 3 mM depending upon the valency of the ions. One set of equal ionic strength solutions, which we have taken in our experiment is 3.0, 1.0 and 0.5 mM for mono-, di- and tri-valent salts, respectively. For obtaining the fluorescence spectra from the heat treated lysozyme and BSA, desired amount of lysozyme and BSA solutions are heated slowly up to 72 °C and then the temperature is reduced up to room temperature (25 °C). Different valent salts were added to the heat treated proteins when it gets the normal room temperature.

For preparing protein films on silicon, silicon substrates were cleaned after keeping in a mixed solution of ammonium hydroxide (NH_4OH, Merck, 30%), hydrogen peroxide (H_2O_2, Merck, 30%) and Milli-Q water (H_2O:NH_4OH:H_2O_2 = 2:1:1, by volume) for 5–10 min at 100 °C. BSA and lysozyme adsorptions were done on silicon after spreading the desired amount of BSA and lysozyme solutions on those substrates and then thoroughly cleaned by the Milli-Q water. Lysozyme and BSA films are also heated slowly up to 72 °C and then the temperature is reduced up to room temperature (25 °C) before dipping into the different desired salt solutions for optical measurements.

UV–vis spectra were taken using Shimadzu UV-1800 UV-Vis spectrophotometer [28]. The fluorescence emission spectra were recorded within a range of 300–600 nm with an excitation wavelength of 273 nm and 271 nm for BSA and lysozyme, respectively. 5 nm slit-widths were used for both excitation and emission monochromators during all the data collection. During fluorescence data collection from protein films, a different sample stage was used to obtain the reflection geometry.

Morphological information of the BSA and lysozyme films before and after interaction with the ions were obtained through an atomic force microscope (NTEGRA Prima, NT-MDT Technology) in semi-contact mode using silicon cantilever having spring constant of 0.03 N/m [28]. Scans were performed over several portions of the films for different scan areas.

3. Results and discussion

Photoluminescence spectra obtained from the lysozyme protein with the concentration variations of the mono-, di- and tri-valent ions are shown in Fig. 1(a)–(c), respectively. Corresponding UV–vis absorption spectra in the presence of different valent ions are