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Regular Article

Insights into the morphology of human serum albumin and sodium dodecyl sulfate complex: A spectroscopic and microscopic approach

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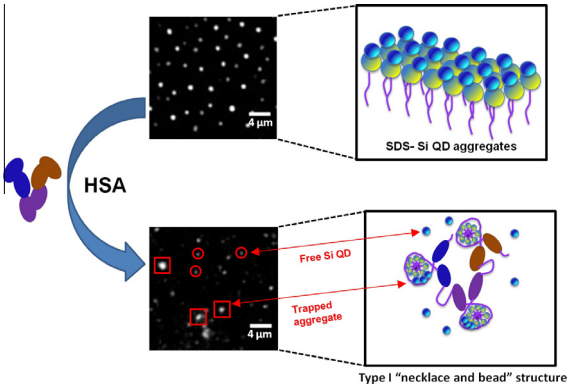
GRAPHICAL ABSTRACT

The morphology and mechanistic insights of complexation between human serum albumin (HSA) and sodium dodecyl sulfate (SDS) have been explored by means of photoluminescence spectroscopy, circular dichroism and PL microscopy using amine-functionalized silicon quantum dot (Si QD) as an external luminescent marker.



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ABSTRACT

Exploring and understanding the fundamental interaction between protein and surfactant is utmost important for various pharmaceutical and biomedical applications. However, very less is known about the arrangement of individual negatively charged sodium dodecyl sulfate (SDS) molecules on the human serum albumin (HSA). Here, we have investigated the morphology and mechanistic insights of complexation between HSA and SDS by means of photoluminescence (PL) spectroscopy, circular dichroism (CD) and PL microscopy using amine-functionalized silicon quantum dot (Si QD) as an external luminescent marker. The present study is based on a unique and dynamic SDS-Si QD system. The synthesized allyla mine–functionalized Si QDs show a distinct PL band centered at 455 nm upon excitation at 375 nm. At neutral pH, these Si QDs form ordered aggregates in the presence of 1 mM SDS due to the hydrogen bonding interaction with the sulfate head groups of surfactants, which is manifested in the appearance of a large Stokes shifted luminescence band centered at 610 nm. It has been observed that the PL intensity of SDS-Si QD aggregates at 610 nm decreases gradually with concomitant increase in the PL intensity of monomeric Si QDs at 455 nm upon increasing the concentration of HSA from 1 to 10 μM. These observations combined with PL lifetime, PL microscopy and CD results reveal that SDS forms micelle-like aggregates on the partially unfolded HSA mainly via electrostatic interaction between negatively charged sulfate head groups and positively charged residues of partially unfolded HSA. For the present HSA-SDS

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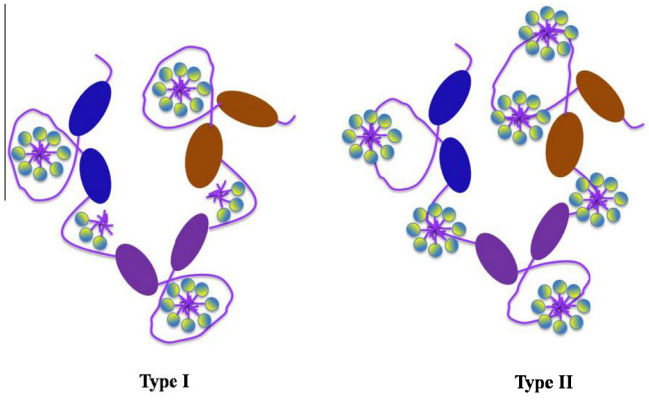
system, our results fit a model with type I "necklace and bead"-like structures, where micelle-like SDS aggregates wrap around by the partially unfolded HSA backbone.

1. Introduction

Over the past few decades, numerous studies have been performed to understand the dynamics, mechanism and morphology of the serum albumin-surfactant complex [1–10]. A variety of techniques such as fluorescence spectroscopy [2–6], nuclear magnetic resonance (NMR) [7,11], light scattering [12–15], small angle neutron scattering (SANS) [16,17], small angle X-ray scattering (SAXS) [18], circular dichroism (CD) [19,20], and surface tension measurement have been applied to unravel the mechanism of protein–surfactant interaction [18]. The well-studied example of protein–surfactant interaction is between serum albumins and sodium dodecyl sulfate (SDS).

Extensive studies have been performed to understand the interaction and subsequent conformational alteration of serum albumins with various surfactants [21,22–27]. Earlier, Moriyama et al. have studied the conformational changes of human serum albumin (HSA) and bovine serum albumin (BSA) in the presence of SDS and dodecyltrimethylammonium bromide (DTAB) surfactants by monitoring the Trp fluorescence signal [22]. Gelamo et al. have reported the interaction of serum albumins with different ionic surfactants and shown that the interaction between HSA and SDS is governed by hydrophobic as well as by electrostatic interactions [23]. Recently, Anand et al. have demonstrated the sequential binding of SDS to 50 μM HSA using intrinsic Trp fluorescence as a marker [21]. It has been proposed that the initial interaction between very low concentrations of surfactants and proteins is predominantly ionic in nature with the surfactant head groups bound to oppositely charged groups on the protein. These initial electrostatic interactions lead to partially unfolded proteins, which subsequently bind with more surfactants in a cooperative fashion and result in the formation of small micelle-like surfactant aggregates on the protein backbone.

These micelle-like surfactant aggregates on the protein backbone are best described by "necklace and bead" model. Earlier, two structurally distinct kinds of "necklace and bead" structures have been proposed for the protein-surfactant complex namely, type I and type II (Scheme 1) [7]. In the type I structure, partially unfolded protein chain wrap around the small micelles-like surfactant aggregates, where the surfactant head groups are in contact with the protein residues due to favorable electrostatic interactions. In contrast, type II structure forms due to the nucleation of small micelles-like surfactant aggregates on the hydrophobic site of the partially unfolded protein chain via hydrophobic interaction, where the hydrocarbon chain of surfactants interact with the hydrophobic sites on the protein backbone and the polar head groups remain free in aqueous solution.



Scheme 1. Two possible structures of "necklace and bead"-like complexes.

Earlier, Oakes studied the binding mechanism between BSA and SDS by means of NMR signal of surfactant molecules and proposed that both the surfactant head groups and alkyl chains interact with the proteins at low surfactant concentrations [11]. However, at higher surfactant concentrations, micelle-like complexes are formed on proteins in which the surfactant alkyl chains are associated with protein apolar residues in a type II "necklace and bead"-like fashion. In contrast, Turro et al. have proposed the formation of type I "necklace and bead"-like complex for BSA-SDS system by using NMR spectroscopy [7]. We have recently demonstrated the first direct microscopic evidence of type II "necklace and bead"-like arrangement of small SDS aggregates on the partially unfolded BSA backbone using amine-functionalized silicon quantum dot (Si QD) as an external luminescence marker through PL spectroscopy and imaging techniques [28]. However, similar microscopic investigation on the structural arrangement of individual SDS molecule on the partially unfolded HSA is rare, and little is known about the overall morphology of HSA-SDS complex at the microscopic level. Although HSA shares 76% sequence similarity with BSA [29], their interaction mechanism differs significantly with several alkyl ligands [19,30]. This motivated us to explore the morphology of the HSA–SDS complex using Si QD as an extrinsic luminescent marker.

Here, we have investigated the morphology of HSA-SDS complex at 1 mM SDS concentration by using Si QD as an external luminescence marker. The present study is based on a unique and dynamic SDS-Si QD aggregated system where the sulfate head groups of SDS are involved in hydrogen bonding interaction with surface amine moieties of Si QDs, while its hydrocarbon chains remain free in solution [31]. This system is unique in the sense that any electrostatic interaction between SDS and HSA will disrupt the ordered aggregates of Si QD while hydrophobic interaction with the hydrocarbon chains of SDS will have minimum perturbation on these aggregates [32]. We have previously shown that this unique binary system has the ability to distinguish the two well known "necklace and bead"-like morphologies of the protein-surfactant (BSA-SDS) and polymer–surfactant complexes [32]. The aim of the present study is to know the arrangement of individual SDS molecules on the partially unfolded HSA backbone.

2. Experimental section

2.1. Materials and sample preparation

Silicon tetrachloride (99%) and tetrahydrofuran (THF, 99.5%) were purchased from Merck (Germany). Allylamine (99%) was purchased from Spectrochem (India). Human serum albumin (HSA, lyophilized powder, essentially fatty acid free), sodium dodecyl sulfate (SDS, 98.5%), tetraoctylammonium bromide (TOAB, 98%), chloroplatinic acid hexahydrate, and isopropyl alcohol (99%) were purchased from Sigma-Aldrich. Lithium aluminum hydride (LAH, 97%) and toluene (99%) were purchased from SD Fine chemicals