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Interaction of a green ester-bonded gemini surfactant with xanthine oxidase: Biophysical perspective

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ABSTRACT

A multi technique approach was utilized to explore the interaction between a novel green gemini surfactant, ethane-1,2-diyl bis(N,N-dimethyl-N-tetradecylammoniumacetoxy) dichloride (14-E2-14), with bovine milk xanthine oxidase (XO). Tensiometric, spectroscopic, microscopic and molecular modeling results demonstrate significant interaction and structural change of native xanthine oxidase upon 14-E2-14 combination. The results obtained in this study may be beneficial for scientists to calibrate conformation of the enzyme by novel biodegradable/green microstructures; consequently, it would likely add new impetus in understanding the treatment modes of various diseases like gout, hyperuricemia, liver and brain necrosis. Moreover, the 14-E2-14–XO interaction assists to unfurl new routes in the designing/selection of green-surfactant–protein mixtures widely used in food processing, cosmetics, and pharmaceutical industries.

1. Introduction

Paramount implications of biomacromolecule–surfactant interactions always made it alluring and fascinating domain for study. These interactions are, therefore, continuing to be under the very active interest of scientists owing to their befitting parlance in almost every sector of industrial world, like pharmaceutics, paints/coatings/adhesives, oil recovery, and so forth [1–4]. Furthermore, protein and surfactant interaction upshots conformational fluctuations of protein in bulk [1,2,5,6] as well at interfaces [7–10]. Therefore, it is appropriate to further interpret the protein–surfactant interactions and their complex formation from scientific as well as practical perspective. Relevance to these studies also lies in unfolding the new ligands (amphiphilic molecules) concomitant with solubilizing, denaturizing [1,2,11] and renaturizing [12–16] properties. Moreover, if cleanliness is next to godliness, then there must be a hefty virtuous dimension to study the protein–surfactant interactions.

Recently, investigations [17–22] have shown that a new class of surfactants, i.e., gemini surfactants, interact with proteins more appreciably than conventional surfactants on behalf of owning outstanding properties viz., lower critical micelle concentration (CMC) and Kraft temperature, special aggregation morphology, strong hydrophobic microdomains, etc. [23–26]. In spite of these promising and captivating properties, studies had predominantly been constrained to implication of quaternary ammonium ion-based cationic *m-s-m* type geminis (where *m* and *s* are the carbon numbers, respectively, in alkyl and spacer chain lengths). Alas! the major flaw associated with these surfactants is their non-cleavability and toxicity [27–29]. Therefore, it seems appropriate to devise and study environmentally benign (novel) surfactants like 14-E2-14, wherein the presence of cleavable weak ester groups (E2) in 14-E2-14 suffices to both legislative pressures and environmental protection demand.

Xanthine oxidase is a complex molybdo-flavo protein. It is a homodimer with each monomer unit composed of three parts: N-terminal domain containing two iron–sulfur centers (Fe/S I and Fe/S II), a central flavin adenine dinucleotide (FAD) domain, and a C-terminal molybdopterin-S binding domain bearing the four redox centers aligned in an almost linear fashion [30–32]. In recent years the enzyme had received an extensive interest by the scientific community owing to its generous application in tissue and vascular injuries, as well as in inflammatory diseases and chronic heart failure [33,34]. In addition, it also catalyzes the oxidation of hypoxanthine to xanthine and that of xanthine to uric acid with simultaneous reduction of molecular oxygen [35]. As is well known, uric acid accumulation results hyperuricemia, gout [36] and related diseases, while as reactive oxygen species generation is coupled

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with the prospective harmful character of the enzyme [34,35,37]. These clinical inadequacies hard-pressed scientists to devise ligands/compounds which can alter the conformation or forage the free radical generated [38,39]. The literature reports [40–42] reveal that a number of metallic and non-metallic compounds have been tested for the cause, but amphiphilic molecules (like 14-E2-14) are yet to be tested, though it is well established that surfactants have healthy capability to bind and denature proteins/enzymes.

Therefore, considering the above mentioned aspects, herein we are reporting the effect of a novel dicationic green ester-bonded gemini surfactant, ethane-1,2-diyl bis(N,N-dimethyl-N-tetradecylammoniumacetoxy) dichloride (14-E2-14), on the structural alterations of bovine milk xanthine oxidase (XO). The interaction was assessed by employing various urbane/authentic techniques viz., tensiometry, fluorescence, absorption spectrophotometry, circular dichroism, transmission electron microscopy (TEM) and molecular modeling. The results obtained are quite appreciative and could assist researchers to calibrate the conformation of an enzyme (xanthine oxidase) which, in turn, can pave the way to understand new routes/methods for the treatment of diseases like gout, hyperuricemia, brain and liver necrosis. Likewise, this study will be prolific to manipulate green-surfactant–protein mixtures in eco friendly industry.

2. Experimental procedures

2.1. Materials

Ethylene glycol (99%, Sigma–Aldrich, USA), chloroacetyl chloride (98%, Loba-chemie, India), N,N-dimethyltetradecylamine (95%, Sigma–Aldrich, USA), bovine milk xanthine oxidase (XO, Sigma–Aldrich, USA), pyrene (98%, Acros Organics, Belgium) were used as received. The green gemini surfactant, ethane-1,2-diyl bis(N,N-dimethyl-N-tetradecylammoniumacetoxy) dichloride (14-E2-14) was synthesized as per Scheme 1. The synthesized product was recrystalized with appropriate solvent mixtures (ethyl acetate/ethanol). ^{1}H NMR and FT-IR [42–46] were utilized for characterization of desired product and the characterization data agreed well with literature. Moreover, purity was further confirmed on the basis of absence of any minimum in the surface tension–concentration profiles [23]. The structure of gemini 14-E2-14 surfactant is given in Scheme 1(b). The concentrations of phosphate buffer and xanthine oxidase throughout the study were 50 mM and 1 μM, respectively.

2.2. Methods

2.2.1. Surface tension measurements

The surface tension measurements were performed on SD Hardson tensiometer (Kolkata, India) at 298 K using ring detachment method. To ensure that there are no protein adsorption effects, the ring (Pt–Ir) was thoroughly rinsed with acetone and dried over alcohol flame. Prior to every experiment the instrument was calibrated. The desired surfactant solution (prepared in 50 mM phosphate buffer) was added to the vessel containing solution of xanthine oxidase (1 μM) in installments, using Hamilton-Bonaduz, SCHWEIZ microsyringe. The surface tension (γ) readings were taken after 10 min of proper mixing.

2.2.2. Fluorescence measurements

Fluorescence probing of 1 μM xanthine oxidase (by varying concentration of 14-E2-14) was recorded on Hitachi F-27000 fluorescence spectrophotometer connected with PC. The fluorescence parameters for intrinsic, extrinsic (pyrene) and three dimensional fluorescence were, respectively, as λ_{ex} = 295 nm, λ_{em} = 300–500 nm, slit width = 5 nm; λ_{ex} = 336 nm, λ_{em} = 350–400 nm, slit width = 10 nm; and λ_{em} = 250–750 nm, slit width = 10 nm. The 14-E2-14 and buffer do not give any signal, hence fluorescence signal can be considered as full contribution of xanthine oxidase. All samples were incubated for 3 min before each measurement.

2.2.3. CD measurements

JASCO-J815 CD spectropolarimeter equipped with microcomputer, thermostatic cell holder and calibrated with D-10 camphor sulfonic acid was employed to probe the conformational changes at 298 K. Requisite solutions of XO and XO + 14-E2-14 were taken in 1 mm path length cell and then far-UV CD spectra (each spectra is the average of three scans) were probed in the range of 200–250 nm with a scan speed of 20 nm/min and response time of 1 s. A reference signal of 14-E2-14 + buffer was subtracted from the CD signal for all measurements.

2.2.4. Absorption spectroscopy

The UV absorption and FT-IR spectra were measured on Perkin Elmer Lambda 25 UV/Visible spectrophotometer and Perkin Elmer FT-IR spectrometer (bearing HATR sampling accessory), respectively. The absorption parameters were as: UV–Vis (200–320 nm, for framework conformation of amide bond), FT-IR (1600–1700 cm^{-1}, to probe amide I bond transitions and 2800–3000 cm^{-1} for micellar phase transitions of the green surfactant).

2.2.5. Transmission electron microscopy

JEM-2100 (JEOL, Japan) electron microscope was employed to carry out TEM measurements. The samples were prepared by casting 1 drop of the pure XO and mixed XO + 14-E2-14 solution onto a copper grid on a glass plate followed by negative staining. The TEM micrographs were then taken after proper sample drying in air (for 1 day).

2.2.6. Molecular docking (computational way to understand XO–14-E2-14 interactions)

Molecular graphics program HEX 6.1 [47] was utilized for molecular modeling of 14-E2-14 in to binding site of xanthine oxidase. It calculates and displays the best fit (lowest energy) docking modes of protein and ligand molecules by employing SPFC (Spherical Polar Fourier Correlations). The HEX 6.1 accepts the ligand and the receptor as input in PDB format. Crystal structure of the bovine milk xanthine oxidase (PDB ID: 1FIQ) was downloaded from the protein data bank (http://www.rcsb.org./pdb). The structure of 14-E2-14 was sketched by using CHEMSKETCH (http://www.acdlabs.com) and converted into PDB format using Chimera 1.9 (www.cgl.ucsf.edu/chimera). Visualization of the docked conformation was achieved employing PyMOL [48] (http://pymol.sourceforge.net). All the programs were run on Intel CORE i5, 2.5 GHz based machine, running MS Windows 7 as the operating system.

3. Results and discussion

3.1. Xanthine oxidase–14-E2-14 interaction (interfacial perspective)

Surface tension is regarded as reliable and effective tool for studying the interactions between biomacromolecules and surfactants. The surface tension curves of pure 14-E2-14 and its mixed system with xanthine oxidase (14-E2-14 + XO) are shown in Fig. 1. The profiles for pure 14-E2-14 comparatively with literature [49,50] shows lower CMC value (0.0077 mM), attribution might be the higher negative dielectrics of solvent medium. Clearly, Fig. 1 suggests that the surface tension (γ) as well as CMC (critical