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Assessing the interaction of Hecameg® with Bovine Serum Albumin and its effect on protein conformation: A spectroscopic study

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ABSTRACT

Interaction of the nonionic surfactant Hecameg® with the plasma protein Bovine Serum Albumin (BSA), and its effect on protein conformation, has been studied using spectroscopic techniques such as steady-state and time-resolved fluorescence and circular dichroism. A weak interaction of the surfactant with BSA is reflected by changes in the intrinsic fluorescence of BSA in either steady-state or time-resolved measurements. The fluorescence intensity data allowed us to determine the corresponding binding curve, which suggests a sequential binding mechanism, in which the surfactant first occupies the hydrophobic sites of the inner protein cavity and then, condenses onto the surface hydrophobic sites of BSA via a cooperative mechanism. Additional fluorescence data obtained by synchronous, three-dimensional and anisotropy experiments show that the surfactant mainly interacts with the tryptophan residues of BSA, which seem to experience motional restriction as a result of this interaction. Time-resolved fluorescence data, which were analyzed using the modified Stern–Volmer equation, also support the above mechanism. Finally, far-UV circular dichroism studies indicated that the secondary structure of the protein remains almost unaltered even for BSA to surfactant molar ratio as high as 1 to 100.

1. Introduction

As a result of their numerous uses in a wide variety of biological, industrial, pharmaceutical and cosmetic applications, the interactions between proteins and surfactants have been extensively studied over the past few decades [1–4], especially as the general principles that govern the binding of surfactants to proteins are analogous to those responsible for the stability of other systems such as bilayers, liposomes or biological membranes [5,6]. Consequently, a better understanding of protein-surfactant interactions is essential from both a fundamental point of view and also because it could help us to choose the most appropriate surfactant for a specific application on a rational basis.

It is well-known that ionic surfactants, particularly anionic ones, interact more strongly with proteins than their neutral counterparts and usually cause major conformational changes in their structure [3,4]. As a result, a great deal of experimental effort has been dedicated to characterizing these interactions [7–29]. Although nonionic surfactants have been shown to be less effective protein-binding agents, recent comprehensive studies on the interaction of such surfactants with water-soluble proteins have also been carried out [30–39]. This is mainly due to the fact that the use of nonionic surfactants is preferred for some applications, especially when it is important to preserve protein stability and functionality. There are two areas in which these aspects are of particular relevance: the stability of protein-based pharmaceutical formulations [34–39] and the isolation and purification of membrane proteins [40,41]. In the former, nonionic surfactants are usually added to both liquid and lyophilized formulations to avoid protein destabilization via adsorption or aggregation mechanisms [39]. The study of membrane proteins requires that they are first extracted from the membrane and then maintained in a soluble, native, and functional form. The usual procedure for achieving this involves solubilization of the membrane protein with surfactants, which is one of the most important steps prior to subsequent biochemical and biophysical studies [40,41].

Alkyl polyglycosides (APGs) are by far the most important family of so-called sugar-based surfactants, which are mainly characterized by having a sugar group as the hydrophilic moiety. These surfactants are currently receiving increasing attention as they are produced from renewable sources, exhibit excellent ecological properties, and are nontoxic and dermatologically safe [42–44]. The unique properties of APGs compared with conventional nonionic ethoxylated surfactants make them very useful in the membrane protein field [41], and their possible use for the preparation of pharmaceutical formulations is being evaluated [45]. In light of this, determination of the binding

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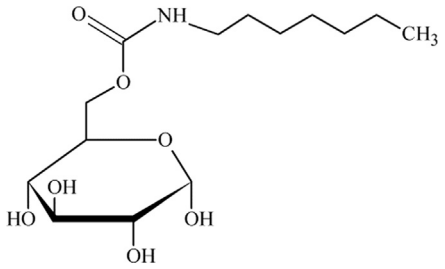

Chart 1. The chemical structure of Hecameg®.

behavior of these surfactants with a model protein is essential as it could provide valuable information regarding the interactions that control this process.

With this in mind, we have carried out a spectroscopic study on the interaction between Hecameg® (6-*O*-(*N*-heptylcarbamoyl)-methyl-α-D-glucopyranoside) and the water-soluble protein Bovine Serum Albumin (BSA). Hecameg® is an APG surfactant with a well-defined amphiphilic structure (see Chart 1), which has long been considered to be a very mild and effective surfactant for biological applications [46,47] and whose aggregation behavior has been well-characterized [48,49]. BSA, an important transporter binding protein, is probably the most widely used and best characterized protein. The popularity of BSA arises due to its high conformational adaptability to a large variety of ligands, including fatty acids, amino acids, metals drugs, surfactants, etc., thus making it a well-established model protein.

2. Experimental

2.1. Materials

Hecameg® (≥ 99%) was purchased from Fluka and BSA (≥ 98%, agarose gel electrophoresis) was obtained from Sigma-Aldrich. Both products were used as received. All other chemicals were of reagent grade and used without further purification. A 20 mM phosphate buffer of pH 7.4 was prepared in ultra-pure water for all experiments. Stock solutions of Hecameg® and BSA were prepared in aqueous buffer solutions and stored at 4 °C. Working solutions with a fixed protein concentration (15 μM) and different concentrations of surfactant were prepared daily, mixed thoroughly, and stabilized for at least 2 h at 25 °C before any spectroscopic measurement was performed. The ultra-pure water (resistivity ~18 MΩ cm^{-1}) used to prepare all the solutions was obtained by passing pure water from a Millipore Elix system through an ultra-high quality Millipore Synergy purification system.

2.2. Instrumentation and methods

2.2.1. Steady-state fluorescence

All steady-state fluorescence measurements were performed using a FluoroMax-4 (Horiba, Jobin Yvon) spectrofluorometer in "S" mode. Sample solutions were placed in a 1 cm path length quartz cuvette. This apparatus is equipped with a 150-W xenon lamp, a Peltier drive to control the temperature in the cell housing to 25.00 ± 0.05 °C, and a polarization accessory and an automatic interchangeable wheel with Glan-Thompson polarizers. An excitation wavelength of 295 nm was applied to selectively excite the tryptophan residues of BSA, with slit widths of 2 nm and 4 nm for excitation and emission, respectively. The degree of anisotropy (r_{ss}) was determined from the fluorescence polarization experiments, all of which were performed using the same apparatus, as follows

$$r_{ss} = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad (1)$$

where the subscripts of the fluorescence intensity values (I) refer to vertical (V) and horizontal (H) polarizer orientation, and G is the instrumental grating factor required for the L-format configuration [50]. The r_{ss} values were averaged over an integration time of 10 s and a minimum number of three measurements were recorded for each sample.

2.2.2. Time-resolved fluorescence

Time-resolved fluorescence measurements were performed using the time-correlated single photon counting technique and a LifeSpec II luminescence spectrometer (Edinburgh Instruments, Ltd.). A nanosecond pulsed light-emitting diode (LED) operating at 295 nm (Edinburgh Instruments, Ltd.) and a pulse period of 100 ns was employed as the excitation source, with emission being recorded at 349 nm. To optimize the signal-to-noise ratio, 10^4 photon counts were collected in the peak channel. The instrumental response function (IRF) was regularly obtained by measuring the scattering of a Ludox solution. The instrumental full width at half maximum (FWHM) for the 295 nm LED, including the detector response, was about 650 ps. The decay curves were deconvoluted using the FAST software package from Edinburgh Instruments. The intensity decay curves for all lifetime measurements were fitted as a sum of exponential terms:

$$I(t) = \sum_i A_i \exp\left(\frac{t}{\tau_i}\right) \quad (2)$$

where A_i is a pre-exponential factor for the component i with a lifetime τ_i. In all cases, the best fit was obtained for a biexponential decay curve, where the quality of the fits was determined by the reduced χ^2 values and the distribution of the weighted residuals among the data channels. The statistical criterion determining the goodness of fit was a χ^2 value ≤ 1.20 and a random distribution of weighted residuals. Average fluorescence lifetimes (τ) were calculated from the two-component contributions using the following equation [50]

$$\tau = \frac{\sum_i A_i \tau_i^2}{\sum_i A_i \tau_i} \quad (3)$$

The relative concentration, or fractional amount of each component (α_i), was determined by:

$$\alpha_i = \frac{A_i}{\sum_i A_i} \quad (4)$$

2.2.3. Circular dichroism

Far-UV circular dichroism (CD) spectra of BSA in the absence and presence of surfactant were recorded using a JASCO 815 spectropolarimeter equipped with a Peltier temperature controller. All CD measurements were performed at 25.0 °C, with a scan speed of 200 nm min^{-1} and a spectral bandwidth of 1 nm. Each spectrum was baseline-corrected, and the final spectrum was taken as the average of five accumulated scans. A dismountable liquid cell with 0.1 cm path length was used, and the spectrometer was continuously purged with dry N_2 gas. For all systems studied, the secondary protein structure was estimated from the corresponding far-UV CD spectrum using the online K2D3 software [51,52].

3. Results and discussion

3.1. Steady-state fluorescence measurements

It is well-known that BSA contains two tryptophan residues (Trp-134 and Trp-212). These residues are characteristic of this protein and are chiefly responsible for the intrinsic fluorescence of BSA. The former (Trp-134) is located in the hydrophilic subdomain