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# Exploring the affinity binding of alkylmaltoside surfactants to bovine serum albumin and their effect on the protein stability: A spectroscopic approach

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ABSTRACT

Steady-state and time-resolved fluorescence together with circular dichroism (CD) spectroscopic studies was performed to examine the interactions between bovine serum albumin (BSA) and two alkylmaltoside surfactants, i.e. *n*-decyl-β-D-maltoside (β-$C_{10}G_2$) and *n*-dodecyl-β-D-maltoside (β-$C_{12}G_2$), having identical structures but different tail lengths. Changes in the intrinsic fluorescence of BSA from static as well as dynamic measurements revealed a weak protein–surfactant interaction and gave the corresponding binding curves, suggesting that the binding mechanism of surfactants to protein is essentially cooperative in nature. The behavior of both surfactants is similar, so that the differences detected were attributed to the more hydrophobic nature of β-$C_{12}G_2$, which favors the adsorption of micelle-like aggregates onto the protein surface. These observations were substantially demonstrated by data derived from synchronous, three-dimensional and anisotropy fluorescence experiments. Changes in the secondary structure of the protein induced by the interaction with surfactants were analyzed by CD to determine the contents of α-helix and β-strand. It was noted that whereas the addition of β-$C_{10}G_2$ appears to stabilize the secondary structure of the protein, β-$C_{12}G_2$ causes a marginal denaturation of BSA for a protein:surfactant molar ratio as high as 1 to 100.



## 1. Introduction

For many years, the interactions between proteins and surfactants have received considerable attention because they play an important role in many technical applications, including areas such as washing, pharmaceuticals, proteomics, cosmetics, and food [1–5]. In addition, these interactions show similarities to those occurring in other surfactant–macromolecular systems [4]. Therefore, a better understanding of the general mechanisms controlling these interactions and how they affect the conformation of the protein is crucial because it can be used as an advantage in designing and choosing novel surfactants.

BSA works biologically as a carrier for fatty acid anions and other simple amphiphiles in the bloodstream. Because of its stability, water solubility, and versatile binding capability, BSA is considered a model protein and has frequently been used for research and as a reference in clinical analyses as well as biochemistry assays [6]. The binding mechanism of surfactants to BSA strongly depends on the surfactant nature. It is well known that anionic surfactants such a sodium dodecyl sulfate (SDS) interact strongly with BSA and other proteins, causing their denaturation [5], and thus much experimental effort has concentrated on characterizing the binding process of SDS and other anionic surfactants to BSA and other globular proteins [7–22]. However, relatively few studies treat cationic or nonionic surfactants or other alternative amphiphilic materials. Recently, studies focusing on the interaction of fluorinated [23–27], gemini [28–34], and ionic liquid surface active agents [34–36] with BSA have appeared.

Because of their ability to absorb onto a variety of substrates and to bind other molecules, the use of globular proteins as functional ingredients in healthcare and pharmaceutical preparations has long been firmly established [37]. Unlike small chemical drugs, proteins show high specificity, which is advantageous compared with other products that target multiple sites. However, only proteins in the native form are active against their target [38]. Consequently, protein stability is today a crucial point to be optimized in the preparation of protein-based drugs [39,40]. The use of surfactants as excipients to avoid structural changes in proteins caused by adsorption is common in the pharmaceutical field [39,40]. In these cases, nonionic surfactants are often preferred over ionic ones as these destabilize protein and exhibit poorer toxicity profiles [41]. As a result, the interaction of nonionic surfactants with globular proteins has also been studied [40–50], though most investigations have focused on the interaction of proteins with conventional nonionic ethoxylated surfactants [41–44,46,49].

Alkylpolyglycosides (APGs), characterized mainly by having one or several glucose molecules in their polar moiety, constitute a relatively

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new class of surfactants which are attracting interest because they can be produced from renewable resources and are biodegradable, nontoxic, and dermatologically safe [51–53]. Because of their mildness and solubilizing power, APG surfactants are receiving considerable attention as feasible alternatives to conventional nonionic ethoxylated surfactants in applications such as the solubilization and purification of membrane proteins [3,54], and drug delivery [55,56]. Consequently, understanding how these surfactants interact with a model protein, and how this interaction affects the protein conformation is essential in order to preserve protein stability and functionality in the aforementioned technical applications. Although a few papers have examined the interaction between APGs with peptides and proteins [45,50,57–59], because of the variability of the APG headgroup structure and the protein nature, detailed studies on the interaction mechanism of specific protein–surfactant systems are still needed.

In this context, we have conducted a spectroscopic study on the interaction of two homologous alkylmaltoside surfactants, *n*-decyl-β-D-maltoside (β-$C_{10}G_2$) and *n*-dodecyl-β-D-maltoside (β-$C_{12}G_2$), with BSA. As an example, Scheme 1 shows the structure of β-$C_{12}G_2$. It can be seen that this structure consists essentially of a double glucose ring attached to a single hydrocarbon chain. That is, the main difference between these surfactants and the conventional nonionic ethoxylated ones resides in the headgroup structure. Whereas the headgroup of the ethoxylated surfactants is flexible and polymer-like, that of maltosides is bulkier and rigid. In fact, these different headgroup structures, together with their different hydration ways, explain the observed differences in their self-assembly behavior [53,60]. The present study has two aims: first, we seek to characterize the binding mechanism and the nature of the interactions underlying in this process, and second, we study how hydrophobicity drives the association between these surfactants and BSA, as both aspects are important in order to establish the basis for a rational choice of surfactants for protein-based pharmaceutical formulations.

## 2. Experimental

### 2.1. Materials

Table 1 lists the surfactants used in the present study, including their origin and (from the literature) the values of their main aggregation characteristics in water. BSA (≥98 %, agarose gel electrophoresis) was obtained from Sigma-Aldrich. Due to their high purity grade, all these samples were used as received. A pH 7.4 phosphate buffer (20 mM) was prepared in ultra-pure water. Stock solutions of surfactants and protein were prepared in aqueous buffer solutions, and then stored at around 4 °C. Working solutions with a fixed BSA concentration and varying surfactant concentrations were prepared daily, mixed thoroughly, and stabilized for at least 2 h at 25 °C before the spectroscopic measurements. The ultra-pure water (resistivity ~18 MΩ·cm) 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. Instruments and spectral measurements

All steady-state fluorescence measurements were made on a FluoroMax-4 (Horiba, Jobin Yvon) spectrofluorometer, equipped with a 150-W xenon lamp and a cell housing with a 1.0-cm path-length quartz cuvettes, which was thermostated by a Peltier drive to 25.00 ± 0.05 °C. This apparatus is also equipped with a polarization accessory and an automatic interchangeable wheel with Glan-Thompson polarizers. To selectively excite the tryptophan residues of BSA, we used an excitation wavelength of 295 nm in all these measurements. The slit widths for excitation and emission were of 2 and 4 nm, respectively. Fluorescence polarization experiments were made to establish the steady-state fluorescence anisotropy, $r_{ss}$, determined as

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

where the subscripts of the fluorescence intensity values ($I$) refer to vertical (V) and horizontal (H) polarizer orientations, and $G$ is the instrumental grating factor required for the L-format configuration [61]. 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.

Fluorescence lifetimes of BSA in the absence and presence of surfactants were determined from time-resolved fluorescence measurements by the method of time-correlated single-photon counting. These measurements were performed using a LifeSpec II luminescence spectrometer (Edinburgh Instruments, Ltd.). The samples were excited by a nanosecond pulsed light-emitting diode (LED) operating at 295 nm (Edinburgh Instruments, Ltd.) and a pulse period of 100 ns, 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 analyzed using the FAST software package from Edinburgh Instruments. The generated curves for intensity decay were fitted as a sum of exponential terms:

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

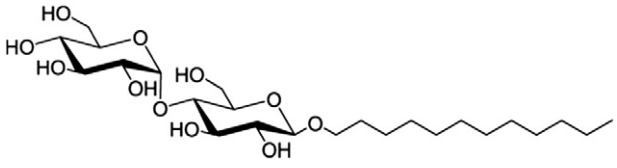
where $A_i$ is a pre-exponential factor for the component $i$ with a lifetime $\tau_i$. In all cases, the best fit was made for a biexponential decay curve, where the quality of the fits was determined by the reduced $\chi^2$ values and the distribution of the weighted residuals among the data channels. The statistical criterion determining the goodness of fit was a $\chi^2$ value ≤1.20 and a random distribution of weighted residuals. The average lifetime, $\tau$, of the protein in each case was calculated using the following equation [61]

$$\tau = \frac{\sum_i A_i \tau_i^2}{\sum_i A_i \tau_i}. \tag{3}$$

And the relative concentration, or fractional amount of each component, $\alpha_i$, was determined by:

$$\alpha_i = \frac{A_i}{\sum_i A_i}. \tag{4}$$

Circular dichroism (CD) was measured with a JASCO 815 spectropolarimeter equipped with a Peltier temperature controller. Far-UV CD spectra de BSA, in the absence and presence of surfactants, were acquired at 25.0 °C, with a scan speed of 200 nm $min^{-1}$ and a spectral bandwidth of 1 nm. A reference sample containing buffer and surfactant was subtracted from the CD signal for all measurements. Each spectrum is 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




**Scheme 1.** The chemical structure of *n*-dodecyl-β-D-maltoside (β-$C_{12}G_2$).