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A combined binding mechanism of nonionic ethoxylated surfactants to bovine serum albumin revealed by fluorescence and circular dichroism

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ABSTRACT

The study systematically investigates aqueous mixtures of fixed bovine serum albumin (BSA) and various ethoxylated nonionic surfactants belonging to a homologous series or not. Mono-disperse tetra-($C_{12}E_4$), hexa-($C_{12}E_6$) and octa-ethyleneglycol mono-n-dodecyl ether ($C_{12}E_8$), and poly-disperse eicosa-ethyleneglycol mono-n-tetradecyl ether ($C_{14}EO_{20}$) are respectively employed. Fluorescence and circular dichroism measurements are performed at surfactant/protein molar ratios (r_m)s lower and higher than one. We aim to get new insights into the binding mechanism of these species and to differentiate among the interaction abilities of these surfactants. The relative magnitude of the binding thermodynamic parameters by fluorescence, and the increase of α-helix prove that hydrogen bonding drives the interaction next to the hydrophobic attraction. $C_{12}E_n$ (n = 4,6,8) develop more H bonds with the albumin than $C_{14}EO_{20}$ owing to a zigzag conformation of their short ethyleneoxide chains. Among the homologous surfactants, $C_{12}E_6$ has a slightly stronger interaction with BSA due to a maximal number of H bonds at a minimal hindering. Static fluorescence and dynamic fluorescence indicate an inter-conversion between the tryptophan (Trp) rotamers which happens around the surfactants critical micellar concentration. For $C_{14}EO_{20}$, the meander conformation of the polar group determines a less evident conversion of the Trp rotamers and smaller α-helix rise. Binding isotherms of the homologous surfactants and the fluorescence quenching mechanism by $C_{12}E_6$ are also provided.

1. Introduction

Serum albumins play an important role in the transport and metabolism of medicines in living organisms. Drug delivery to target requires biocompatible carriers, a condition successfully accomplished by nonionic micelles [1–3]. Unlike ionic surfactants, the neutral counterparts have weaker interactions with albumins [4], do not denature and stabilize the peptides' secondary structure [5]. The interaction between serum proteins and nonionic surfactants has mostly been regarded as hydrophobic, with a more important binding of the alkyl chains than of the headgroups [4]. Sjögren and coauthors showed that model peptides with a pronounced hydrophilic character do not change their conformation in the presence of nonionic surfactants [5]. However, the large, negative enthalpy of reaction for mixtures of bovine serum albumin (BSA) and hepta-($C_{12}E_7$) or pentaethyleneglycol mono-n-dodecyl ether ($C_{12}E_5$) could not be solely attributed to the hydrophobic attraction [6]. An earlier review on bioactive compounds and alkyl ethoxylated surfactants took into consideration besides the hydrophobic attraction, eventual electrostatic forces and hydrogen bonds among the more polar amino acids and the ethyleneoxide (EO) chains, and the phenomenon depended considerably on the molecular species involved [7]. The complex formation between BSA and poly(ethyleneglycol) (PEG) was explained by the possible occurrence of H bonding next to the hydrophobic attraction [8,9]. Hydrogen bonds were also supposed to exist in the interaction of serum albumins with different compounds containing a few oxygen atoms in their molecules [10–13]. Molecular dynamics simulations on BSA and Tween 20 or Tween 80 have recently proved that the hydrophilic and hydrophobic groups of the albumin equally contribute to the area of interaction with the surfactant [14].

On the other hand, it is well documented that depending on solubility, a drug may locate within a micellar system near or at the micelle surface, between the hydrophilic groups and the palisade layer, in the palisade layer or in the core [15]. The size of the hydrophilic moiety is therefore important for drug accommodation and it makes sense to investigate its role in the albumin–surfactant interaction. Tetra-($C_{12}E_4$), hexa-($C_{12}E_6$) and octaethyleneglycol mono-n-dodecyl ether ($C_{12}E_8$) belong to a homologous series having the same hydrophobic tail but various, relatively short and homogeneous EO chains. To the opposite, eicosa-ethyleneglycol mono-n-tetradecyl ether ($C_{14}EO_{20}$) has a much longer and polydisperse EO chain and a larger hydrophobic moiety. To the best of our knowledge their interaction with albumins, whether has been studied was essentially regarded as hydrophobic [4,16].

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Our study systematically investigates $C_{12}E_4$, $C_{12}E_6$, $C_{12}E_8$ and as a counter-case, $C_{14}EO_{20}$ in mixtures with BSA, aiming to achieve new insights into the binding mechanism and to distinguish among their interaction abilities. Unlike the majority of studies that deal with nonionic surfactant/protein molar ratios (r_m) higher than one [6,17,18], we consider as well the $r_m < 1$ case and surfactant concentrations much higher than the critical micellar concentration (CMC). Spectroscopic data evidence the hydrogen bonding as another driving force of interaction that protects the BSA helical structure. The results reveal an optimum EO chain length for hydrogen bonding with minimal hindering in case of $C_{12}E_6$. The results have potential applicability in developing drugs with enhanced delivery capacity.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA), Fraction V, for biochemistry, was purchased from Merck having a purity > 98% (agarose gel electrophoresis) and used as received. It was essentially globulin-free and had a fatty acid content < 0.2%, as declared by the producer. The protein was used at a fixed concentration of 8.5×10^{-7} M in all the measurements. The working concentration was freshly prepared by aqueous dilution of BSA (10^{-4} M) in Phosphate Buffer Saline. The exact BSA concentration was spectrophotometrically determined in phosphate buffer saline of pH = 7.4 by using a molar extinction coefficient of 43,824 M^{-1} cm^{-1} at 280 nm [19].

Human serum albumin (HSA) was purchased from Sigma-Aldrich and used without purification. It has purity of 99% (agarose gel electrophoresis) declared by the producer in the batch certificate of analysis. HSA was used for comparison in similar experimental conditions as BSA.

Mono-disperse tetra-($C_{12}E_4$), hexa-($C_{12}E_6$) and octa-ethyleneglycol mono-n-dodecyl ether ($C_{12}E_8$) were supplied from Sigma-Aldrich having purities of 99.2% (GC), 100% (TLC) and respectively, 100% (TLC) as declared in the certificates of analysis of the producer. Poly-disperse eicosa-ethyleneglycol mono-n-tetradecyl ether ($C_{14}EO_{20}$) was supplied by Nikkol Chemical (Tokyo, Japan). Its CMC was determined using Pyrene (Py) to probe the micro-polarity of $C_{14}EO_{20}$ aqueous solutions, before and after micelles formation. The breaking point in the variation of Py polarity ratio (I_I/I_{III}) with surfactant concentration was taken as the CMC (see Fig. S1 in the Supplementary Information). All the surfactants were utilized as received. Within the experiments, the surfactants were used in aqueous mixtures with serum albumins, at surfactant/protein molar ratios (r_m) lower and higher than 1. The solutions were prepared with Millipore water.

2.2. Methods

The exact BSA concentration was spectrophotometrically determined with a Carry 100 Bio spectrophotometer.

The pH of the BSA–surfactant mixtures was checked at 25 °C, with an Orion 420 A pH-meter calibrated with standard buffers.

Static fluorescence measurements were performed in thermostatic conditions (with a precision of ±0.01 K) on a Horiba-Jobin-Yvon spectrofluorometer. The water Raman spectrum was periodically checked and indicated a peak higher than 400,000 cps at 397 nm, as the producer recommends. The absorbances of the BSA–surfactant samples were below 0.05, so the inner filter effect was avoided and the fluorescence correction was unnecessary [20–22]. The emission spectra were registered in between (305–550) nm and (295–500) nm for the excitation wavelengths of 295 nm and 278 nm, respectively. The slits were set at 4 nm for excitation and at 3 nm for emission. Variable temperatures (288, 293, 298, 303 and 309 K) were considered for the emission spectra registration. To allow a discriminatory judgment, static fluorescence measurements were performed at 298 K on HSA–$C_{12}E_6$ mixtures, in similar experimental conditions as for the BSA-counterpart.

Fluorescence lifetime determinations were performed with an Edinburgh Instruments F920 spectrofluorometer. The measurements were carried out at 298 K using the time-correlated single photon counting technique. The excitation wavelength was 295 nm and the fluorescence emission was collected at 345 nm. The instrumental diffusion was measured using a Ludox solution. The time-dependent fluorescence intensity was fitted to a model with two or three exponentials using the Marquardt–Levenberg algorithm. The goodness-of-fit criterion (χ^2) and the residual curve were checked each time in order to obtain reliable lifetime values. A minimal value for χ^2, in between 1 and 1.35, and residual data looking like random noise around zero were accepted.

Circular dichroism (CD) measurements were done at 298 K with a Jasco J-815 Spectropolarimeter, equipped with a Peltier temperature controller. For each CD spectrum, an average of 3 scans was recorded in the range of 190–290 nm. CD data were expressed in terms of mean residue ellipticity (MRE) in deg·cm^2·$dmol^{-1}$ [23] and the secondary structure contents were estimated using the online platform DichroWeb (analysis program CDSSTR, reference set 7) [24,25]. The fittings had normalized root mean-square deviations (NRMSD) lower than 0.1.

The percent of α-helix was also determined from the dichroic spectra using the relations (1) and (2) [26,27]:

$$\alpha\text{-}helix(\%) = \frac{[-MRE_{208} - 4000]}{33,000 - 4000} \times 100 \quad (1)$$

where MRE_{208} represents the mean residual ellipticity at one of the characteristic minima for α-helices (208 nm), calculated with formula (2):

$$MRE = \frac{observedCD}{C_p \times n \times l \times 10} \quad (2)$$

in which *observedCD* means the circular dichroism (at 208 nm in this case), C_P is the protein molar concentration, n is the number of amino acids in the protein chain (583 for BSA) and l is the path length (1 cm). In relation (1), 4000 and 33,000 respectively stand for MRE_{208} for random coil and pure α-helix conformation.

All the fluorescence and CD spectra were recorded using cells of 1 cm path length. An incubation time of 1 h was kept for albumins before each type of measurement.

3. Results and Discussion

3.1. Static Fluorescence

The intrinsic fluorescence of BSA comes mostly from two tryptophan (Trp) residues, with only a minor contribution from the numerous tyrosines. The emission of Trp is the only one highly sensitive to the modifications in the local environment, indicating protein conformational changes, binding to substrates and denaturalizing [28]. The two Trp residues of BSA are respectively located in the first homologous domain at the protein surface (Trp 134), and in the second domain, within a hydrophobic pocket (Trp 212) [29]. Emission spectra for BSA, with and without various concentrations of ethoxylated surfactants, were taken at $\lambda_{exc} = 278$ nm and at $\lambda_{exc} = 295$ nm. The former excitation wavelength corresponds to BSA absorption maximum and gives the global fluorescence, including the tyrosines signal. The spectra present a large maximum around 345 nm, corresponding to the composed signals of Trp and tyrosine (data not shown). The latter excitation wavelength ($\lambda_{exc} = 295$ nm) was chosen to excite solely the tryptophan residues and the emission spectrum of 8.5×10^{-7} M aqueous BSA shows a maximum at 345 nm and a shoulder around 330 nm. Literature data illustrated as well a composed fluorescent signal at small albumin concentration and $\lambda_{exc} = 295$ nm, but the authors didn't make any assignment [30]. On the other hand, emission spectra at $\lambda_{exc} =$