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Saponin Adsorption at the Air−Water Interface—Neutron Reflectivity and Surface Tension Study

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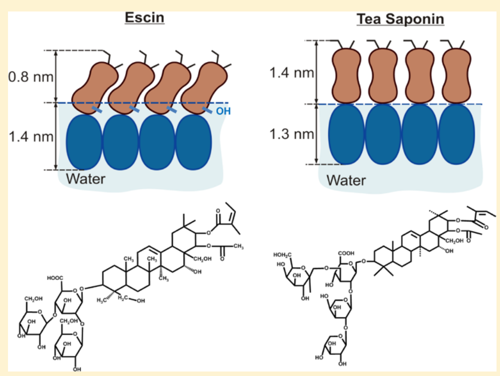
Supporting Information

ABSTRACT: Saponins are a large group of glycosides present in many plant species. They exhibit high surface activity, which arises from a hydrophobic scaffold of triterpenoid or steroid groups and attached hydrophilic saccharide chains. The diversity of molecular structures, present in various plants, gives rise to a rich variety of physicochemical properties and biological activity and results in a wide range of applications in foods, cosmetics, medicine, and several other industrial sectors. Saponin surface activity is a key property in such applications and here the adsorption of three triterpenoid saponins, escin, tea saponins, and *Quillaja* saponin, is studied at the air−water interface by neutron reflectivity and surface tension. All these saponins form adsorption layers with very high surface visco-elasticity. The structure of the adsorbed layers has been determined from the neutron reflectivity data and is related to the molecular structure of the saponins. The results indicate that the structure of the saturated adsorption layers is governed by densely packed hydrophilic saccharide groups. The tight molecular packing and the strong hydrogen bonds between the neighboring saccharide groups are the main reasons for the unusual rheological properties of the saponin adsorption layers.



■ INTRODUCTION

Saponins are a large group of glycosides present in more than 500 plant species.[1−4] Their intrinsic surface activity distinguishes them from other glycosides. This surface activity arises from a hydrophobic scaffold comprising a triterpenoid, steroid, or steroid−alkaloid group and a hydrophilic part consisting of saccharide residues, attached to the hydrophobic scaffold via glycoside bonds. The wide range of different molecular structures of the saponins, found in various plant species, gives rise to a rich variety of physicochemical properties and biological activity.

The intrinsic surface activity of the saponins is the basis of their traditional use as emulsifiers and foaming agents in foods[5,6] and their biological properties are recognized in natural medication.[7,8] More recently, the surface and biological activities have resulted in the identification and development of a variety of new applications in medicine, foods, and cosmetics. Saponins are currently used as foam and emulsion stabilizers in beer and soft drinks,[2,6] and as solubilizing agents for food additives.[9] They are used as stabilizers in cosmetic emulsions, shampoos, and conditioners, and in skin antiaging products.[10] Saponins possess anti-inflammatory, antifungal, antibacterial, anticancer, and cholesterol lowering functions.[11] These properties make the saponins potentially important for an even wider range of new applications and technologies. Hence, some aspects of the adsorption behavior of saponins,[12−16] saponin/surfactant,[17] and saponin/protein[18−21] mixtures have been studied recently, and their self-assembly in solution has been characterized.[22,23]

The saponins exhibit some very unusual surface properties, such as an extremely high surface modulus and shear visco-elasticity. These properties have an important impact on the mechanisms associated with foam and emulsion stabilization by these molecules. Hence, several detailed studies have been focused recently on the surface rheological properties of saponin solutions.[12−15] Stanimirova et al.[12] and Golemanov et al.[13] showed that the *Quillaja* saponin adsorption layers, subjected to small deformations, exhibit a very high surface dilatational elasticity, ∼280 mN m^{-1}, lower shear elasticity, ∼26 mN m^{-1}, and a negligible dilatational viscosity. The high

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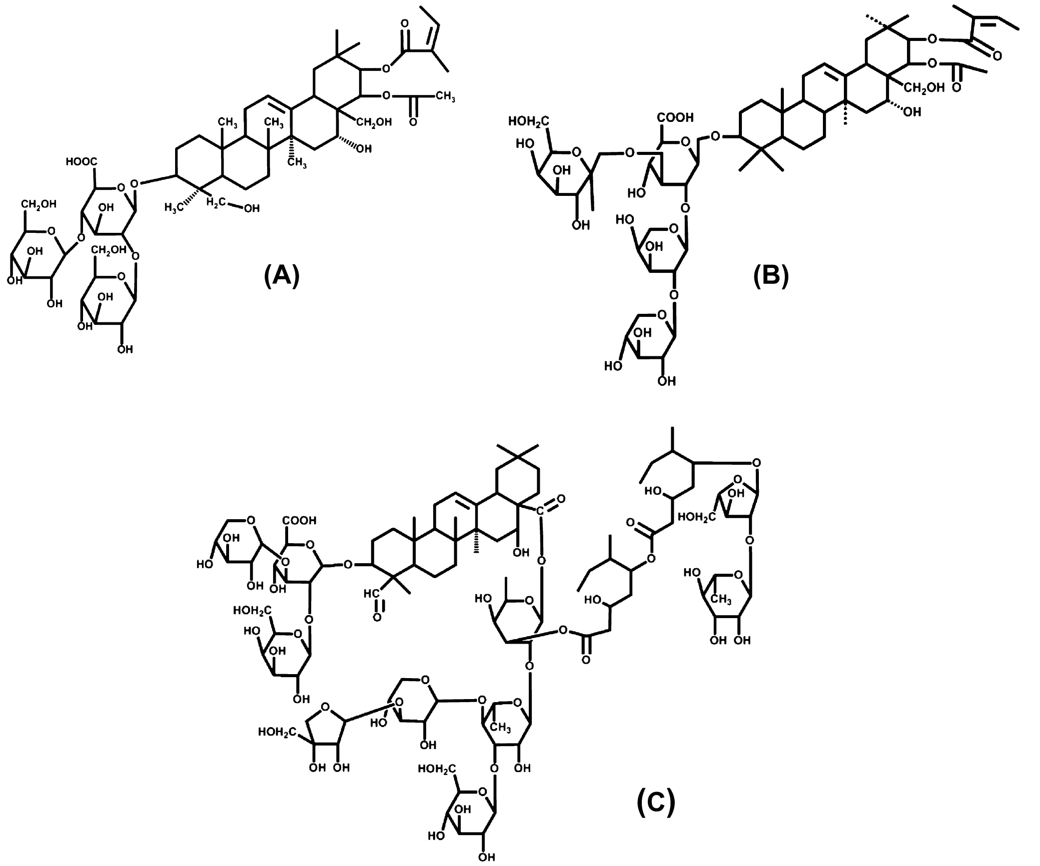

Figure 1. Molecular structure of the saponins studied, (A) escin, (B) tea saponin, and (C) *Quillaja* saponin. The escin and tea saponin have only one oligosaccharide chain, attached to the triterpenoid scaffold, but differ in the number and the type of sugar residues. The *Quillaja* saponin is a larger molecule with more sugar residues, combined in two separate oligosaccharide chains.

surface elasticity is indicative of densely packed solid-like adsorbed layers, with strong intermolecular interactions, arising from multiple hydrogen bonds between neighboring sugar groups. Golemanov et al.[13] showed that the surface layers of the *Quillaja* triterpenoid saponins were highly elastic, whereas the steroid-based *Yucca* saponin layers were purely viscous. In a subsequent paper, Golemanov et al.[14] and Pagureva et al.[15] studied the surface rheology of a wider range of triterpenoid and steroid saponins and showed that all steroid saponins exhibited no shear elasticity and had negligible surface viscosity. In contrast, most of the triterpenoid saponins showed complex visco-elastic surface behavior with extremely high surface elastic modulus (up to 1100 mN/m) and surface viscosity (130 N·s/m). The saponin extracts, showing the highest elastic moduli, were those of escin, tea saponins and berry saponins, all containing predominantly monodesmosidic triterpenoid saponins (with a single oligosaccharide chain attached to the triterpenoid group). Hence, to explain the reasons for the unusually high visco-elasticity of the adsorption layers observed in some saponins, it is important to characterize these adsorbed layers in more detail. Both the total adsorbed amount and the structure of the adsorbed layer will provide important information about the interactions between adsorbed saponin molecules.

Hence, three different saponins, escin, tea saponins, and *Quillaja* saponin, were studied and are reported in this paper. The molecular structures of these saponins are shown in Figure 1. These are all triterpenoid saponins which exhibit rather high surface visco-elasticity. The main difference between these saponins is in the number and length of the oligosaccharide hydrophilic chains. Escin and tea saponins have only one oligosaccharide chain attached to the triterpenoid group (monodesmosidic saponins) with tea saponin having one more saccharide group, whereas the *Quillaja* saponins have two oligosaccharide chains attached to the triterpenoid group (bidesmosidic saponin).

It is now well established that neutron reflectivity can be used to determine adsorption at interfaces[24] and has been demonstrated for surfactants,[24] polymers,[25] proteins,[26] and their associated mixtures at the air–water interface. Although isotopic substitution, using H/D exchange, is often used to optimize the selectivity and sensitivity of neutron reflectivity to the adsorption, it has been shown that for proteins and some other large biomolecules, there is often sufficient contrast without the need to use deuterium labeling.[26,27] This is particularly important here in the context of studying the adsorption of saponin at the air–water interface, where the composition and size of the saponin molecules ensures a sufficient contrast to observe their adsorption at the air–water interface using neutron reflectivity without the need for deuterium labeling. However, deuterium labeling or partial labeling of the solvent can help to provide additional structural in-sights, as demonstrated in this study.

Measurements were made at the air–water interface, at concentrations from below to above the critical micelle concentration (cmc) of the saponins to establish the adsorption isotherms. Neutron reflectivity data were obtained and surface tension measurements made to quantify the amount of adsorbed saponin. For escin and tea saponin above the cmc, neutron reflectivity measurements were made with different solvent contrasts, in D_2O and H_2O/D_2O mixture, to determine in more detail the structure of the adsorbed layer. For escin, the effect of pH on the adsorption was also studied, as it contains a carboxylic group in one of its saccharide groups. The neutron reflectivity and surface tension measurements were made on the same systems and under the same solution conditions.

EXPERIMENTAL SECTION

Materials Used. The escin, also known as aescin, was obtained from Sigma (cat. no. E1378, CAS number 6805-41-0, molecular formula $C_{54}H_{84}O_{23}$). The tea saponin extract was obtained from Zhejiang Yuhong Import & Export Co., Ltd, and used as supplied. According to the product data sheets, the concentrations of saponins in these extracts were 95% for escin and 96.2% for the tea saponin. The *Quillaja* saponin extract (Supersap) was obtained from Desert King, Chile. It is a white powder produced for pharmaceutical applications, with very high purity, and contains 91 wt % saponins. The rest is ~8 wt % moisture and traces of electrolytes and other organic ingredients. As such only escin is a monomolecular substance. The tea saponin and Supersap extracts studied contain a variety of molecular species, which share the same triterpenoid scaffold but differ to some extend in the number and type of the attached saccharide groups. In Figure 1 we show and in the following consideration we use the predominant component in the respective extracts as representative for the whole extract.

All solutions contained 10 mM NaCl to maintain a fixed ionic strength. The pH of the working solutions was adjusted by adding small aliquots of 0.1 M NaOH or 0.1 M HCl solutions, prepared from NaOH with purity ≥95 and 32% HCl acid (both products of Sigma-Aldrich).

Experimental Methods. The neutron reflectivity measurements were made on the INTER reflectometer at the ISIS Neutron Source.[28] The neutron beam was incident at the air–water interface with a grazing angle of incidence, $\theta = 2.3°$, to cover a wave vector transfer, $Q = 4\pi \sin \theta/\lambda$, in the direction normal to the surface in the range between 0.03 and 0.5 Å^{-1}, using neutron wavelengths from 0.5 to 15 Å. The reflectivity, $R(Q)$, was calibrated with respect to the direct beam intensity and reflection from a D_2O surface. The measurements were made in sealed Teflon troughs with sample volumes ~25 mL and maintained at a temperature of 25 °C. Each reflectivity profile took ~60 min, and repeated measurements over a 3–4 h period showed no further changes with time. Hence, it is assumed that the data presented represent equilibrium adsorption values.

In the kinematic or Born approximation, the reflectivity $R(Q)$ is related to the square of the Fourier transform of the scattering length density profile, $\rho(z)$[24]

$$R(Q) = \frac{16\pi^2}{Q^2}\left|\int \rho(z)\, e^{-iQz}\, dz\right|^2 \tag{1}$$

where $\rho(z) = \sum_i n_i(z) b_i$, $n_i(z)$ is the number density of the i-th component and b_i is its scattering length. The scattering length, molecular weight, and molecular volumes of the three saponins studied are listed in Table 1. However, in the context of this study the standard optical matrix method is used to analyze the data.[24]

Table 1. Saponin Parameters Used for Analysis of Neutron Reflectivity Data

saponin type	$\sum b$ ($\times10^{-4}$ nm)	molecular weight	ρ ($\times10^{-8}$ nm^{-2})
escin	1.78	1101	1.6
tea	1.97	1259	1.5
Quillaja saponin	2.00	1650	1.2

The adsorption measurements were made in null reflecting water, nrw, (92 mol % H_2O/8 mol % D_2O) with a scattering length density of zero, the same as air. The data are consistent with a single monolayer at the interface. Analyzing the data as a single layer of uniform composition yields a layer thickness, d, and a scattering length density, ρ. The area/molecule is then given by

$$A = \sum b_i/\rho d \tag{2}$$

and the adsorbed amount, Γ [mol·m^{-2}], is then

$$\Gamma = 1/N_a A \tag{3}$$

where N_a is the Avogadro number. Additional neutron reflectivity measurements were made for the escin and tea saponins at a concentration higher than the cmc in D_2O and a D_2O/H_2O mixture, index matched to $\rho = 4 \times 10^{-8}$ nm^{-2}, to obtain a more detailed description of the adsorbed layer structure. From this sequence of measurements, the data were simultaneously analyzed with the simplest model consistent with the data, in this case a two-layer model. In both the adsorption measurements and the more detailed structural measurements, the key refinable model parameters are the thickness and scattering length density of the layers and a flat sample/instrumental background (~5×10^{-6} to 8×10^{-6}). In both single- and two-layer characterizations, the simplest model consistent with all of the data is used; and additional parameters, such as roughness which can be quite arbitrary and difficult to assign and physical significance to, are not included.

The Wilhelmy plate method[29,30] was used to determine the equilibrium surface tension of the saponin solutions, σ_e. These measurements were made on a K100 tensiometer (Krüss GmbH, Hamburg, Germany) using a platinum plate. Before each measurement, this plate was cleaned by heating on a flame, followed by abundant rinsing with deionized water. The measurements were all made at a constant temperature of 20 °C.

RESULTS AND DISCUSSION

Neutron Reflectivity Data. The adsorption of the escin saponin was measured at the air–water interface in nrw, in the concentration range of 2×10^{-6} to 1.5×10^{-3} M/L by neutron reflectivity. The data are modeled as a single uniform layer with a thickness of ≈2.6 nm. The variation in the adsorbed amount with the surfactant concentration, at natural pH, pH 4, and pH 8, is shown in Figure 2. The saturated adsorbed amounts at the different pH values are evaluated as 2.2, 2.4, and 2.5 ± 0.3 μmol·m^{-2} at pH 8, natural pH, and pH 4, respectively; corresponding to area/molecule of 0.75, 0.69, and 0.61 ± 0.04 nm^2. However, within experimental error, the adsorption at

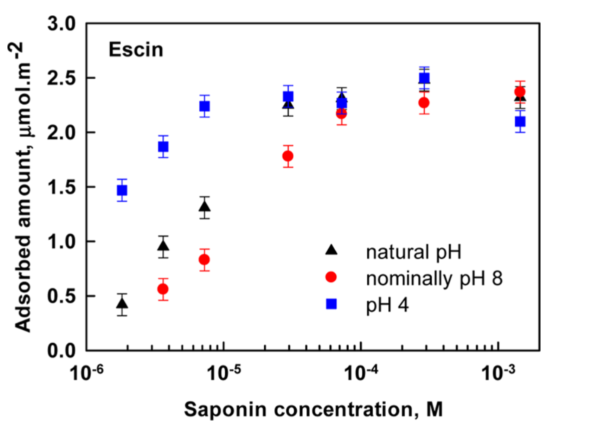

Figure 2. Adsorbed amount vs surfactant concentration at the air–water interface for escin saponin at natural pH (black triangles), pH = 8 (red circles), and pH = 4 (blue squares).

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and above the cmc is independent of pH and has a mean value ≈2.4 ± 0.3 μmol·m^{-2} (area/molecule ≈ 0.69 ± 0.04 nm^2). A clear effect of changing the solution pH is evident at lower saponin concentrations and as the pH decreases from 8 to 4, the onset of adsorption saturation occurs at about a fivefold lower concentration.

The latter results indicate that escin does not behave entirely as a simple nonionic surfactant. This is due to the presence of a carboxylic group in one of its sugar groups, see Figure 1A. The carboxylic group is expected to be protonated at pH = 4, ionized at pH = 8, and probably, it is partially ionized at the natural pH, which varies between ≈5.2 and ≈4.7 at a saponin concentration between 1.82 and 7.26 μmol/L. Nevertheless, as discussed above, the saturation saponin adsorption is, within experimental error, not affected by pH. The results shows that the saturation adsorption is controlled by the molecular dimensions which determine the area per molecule at close packing in the layer, while the presence of the ionic charge has a major effect in diluted adsorption layers only. The triterpenoid scaffold in the saponin molecules is rather large (it contains 30 carbon atoms) and, therefore, the surface activity of these molecules remains very high, even in the presence of ionic charge.

Similar neutron reflectivity measurements were made in nrw, at natural pH only, for the tea saponin and for *Quillaja* saponin (Supersap) in the concentration range between 10^{-3} and 0.5 mM. The respective saponin adsorption isotherms are shown in Figure 3 and compared with that measured for escin at natural pH. The mean adsorption for the tea saponin above the cmc is ≈2.1 μmol·m^{-2} (area/molecule ≈ 0.79 ± 0.04 nm^2) and for Supersap it is ≈1.9 μmol·m^{-2} (area/molecule ≈ 0.87 ± 0.0 nm^2). As expected, the area per molecule at saturation increases with the molecular mass of the saponin molecules. The surface saturation occurs at comparable bulk molecular concentrations of escin and tea saponins because their hydrophobic fragments are very similar, see the comparison in Figure 1A,B. The surface saturation occurs at a much lower concentration for Supersap because of the additional hydrophobic chain present in bidesmosidic *Quillaja* saponin molecules, see Figure 1C, which leads to higher surface activity. However, given the lower level of impurity in this saponin, further more detailed structural measurements were not pursued here.

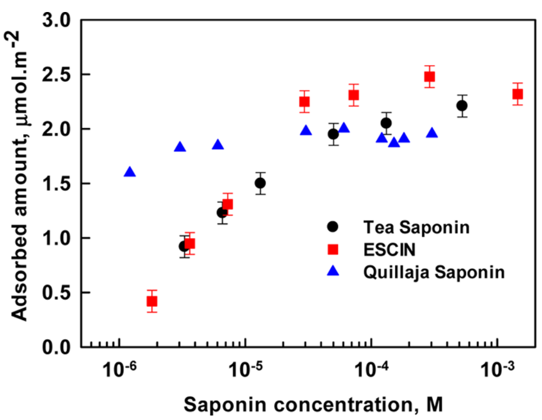

Figure 3. Adsorbed amount vs surfactant concentration at the air—water interface for tea saponin (black circles), escin saponin (red squares), and *Quillaja* saponin (blue triangles) at natural pH.

To determine the total adsorbed amount from the neutron reflectivity data, the product $d \cdot \rho$ is sufficient, see eq 2. For the escin and tea saponins, the data at saturation adsorption were further analyzed by systematically varying d to obtain a more accurate estimate of the thickness of the adsorbed layer in the frame of the homogeneous monolayer model. The variation in X^2 ("goodness of fit") with d provides a more accurate evaluation of the monolayer thickness and its associated error. This is shown in Figure S1 for escin and tea saponin in the Supporting Information. By minimizing the variation in X^2 values the mean adsorbed layer thickness of 2.8 ± 0.2 nm for escin and 3.0 ± 0.2 nm for the tea saponin were derived. A more detailed characterization of the structure of the adsorbed layer for the escin and tea saponins was made by making a series of neutron reflection measurements with different solvent contrasts, in nrw, D_2O, and a D_2O/H_2O mixture with scattering length density ≈4 × 10^{-8} nm^{-2} (cm4.0). This latter scattering length density was estimated to be intermediate between that for D_2O and the hydrated headgroup region and should provide the greatest sensitivity to the structural details in combination with the measurements in nrw and D_2O. The experimental data, obtained with 0.3 mM escin solutions (>cmc), at natural pH, are shown in Figure 4a along with the associated model fits. The data for the different contrasts were analyzed simultaneously using the same structural model, in which only the scattering length densities of the layers were independent variables. The single-layer

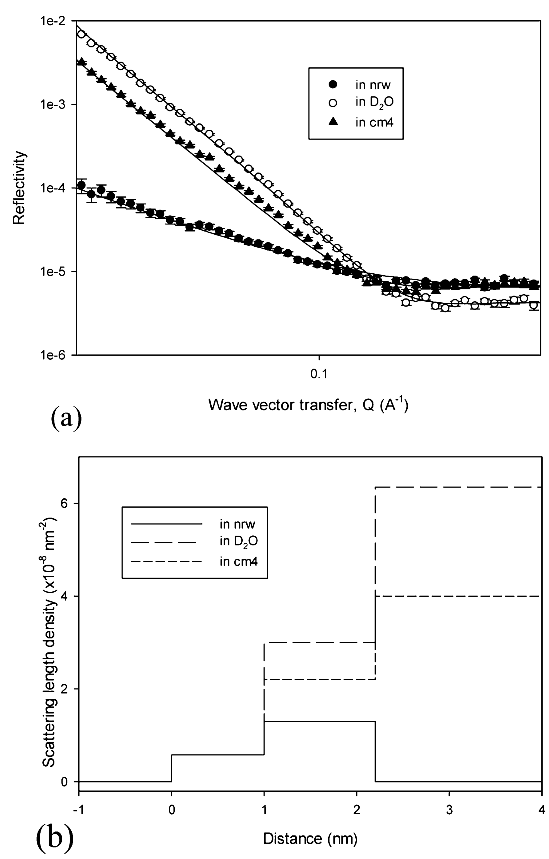

Figure 4. (a) Neutron reflectivity profile for escin saponin at 0.3 mM in nrw, D_2O, and cm4.0 solvents. The solid lines are model fits using the profiles in b; (b) scattering length density profiles from model fits, (—) nrw, (...) cm4.0, and (--) D_2O, for key model parameters summarized in Table 2.

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Table 2. Key Model Parameters from Analysis of Neutron Reflectivity in Figures 4a and S2a

saponin type	concentration (mM)	solvent contrast	d_1 [±0.1 nm]	ρ_1 (±0.04 × 10^{-8} nm^{-2})	d_2 [± 0.1 nm]	ρ_2 (±0.04 × 10^{-8} nm^{-2})
escin	0.3	nrw	0.8	0.58	1.4	1.3
escin	0.3	D_2O	0.8	0.58	1.4	3.0
escin	0.3	cm4.0	0.8	0.58	1.4	2.2
tea	0.13	nrw	1.4	0.7	1.3	1.0
tea	0.13	D_2O	1.4	0.7	1.3	2.8
tea	0.13	cm4.0	1.4	0.7	1.3	2.8

model, used for the data in nrw to estimate the adsorbed amount, was not sufficient when the solvent contrast was altered and the different regions of the surface were highlighted differently. The simplest model consistent with these data was a two-layer model. The corresponding scattering length density distributions are shown in Figure 4b, and the associated parameters are summarized in Table 2.

The results in Table 2 show an outer layer, adjacent to the air phase, with thickness ~0.8 nm, volume fraction of saponin ~0.3, and no solvent. This outer layer corresponds to the triterpenoid hydrophobic region of the saponin molecules. Taking into account that the total length of this hydrophobic region is ≈1.4 nm, we interpret this result as an indication that a fraction of this scaffold is embedded in the aqueous subphase and/or that the triterpenoid scaffold is strongly tilted with respect to the air−water interface. Both these explanations are consistent with a recent simulation of escin adsorption by molecular dynamics.[31] The inner layer, adjacent to the aqueous phase, is ≈1.4 nm thick. It contains both saponin and water, with volume fractions of ≈0.8 and 0.2, respectively. The high volume fraction of the saponin fragments in this inner layer (80 vol %) reveals that it is composed mostly of tightly packed, hydrated sugar groups.

A similar sequence of neutron reflection measurements was made for the tea saponin at natural pH and a saponin concentration of 0.13 mM (above cmc). The neutron reflection data and the corresponding model fits are shown in Figure S2a in the Supporting Information. The corresponding scattering length density profiles are shown in Figure S2b, and the key model parameters are listed in Table 2. The two-layer model is also the simplest one consistent with the data and is different to that observed for escin. The outer layer, adjacent to the air phase is ≈1.4 nm thick, has a saponin volume fraction ≈0.4, and contains no solvent. The inner layer, adjacent to the aqueous phase, is ≈1.3 nm thick, has a volume fraction of saponin ≈0.65, and a corresponding water volume fraction ≈0.35.

Figure 5 presents schematically the structure of adsorption layers of escin and tea saponin. These results indicate qualitatively similar structures and packing of the escin and tea saponin molecules in their adsorption layers, however, with some noticeable differences. The thickness of the solvated hydrophilic region is similar for both saponins, and the slight difference in the saponin volume fraction in that inner layer reflects the higher adsorption of the escin compared with tea saponin. For both saponins, the volume fraction in the inner layer is relatively high. This is consistent with the strong intermolecular interactions which arise from multiple hydrogen bonding between neighboring sugar groups and which are assumed to be responsible for the high surface elasticity, observed with these adsorption layers.[12−14] The most important difference between the two saponins is that the hydrophobic part of the tea saponin adsorption layer is much thicker and seems to be composed of triterpenoid scaffold which is directed perpendicularly to the surface and is entirely exposed to the air phase. Whether this difference is due to the extra hydroxyl group present in the triterpenoid part of the escin (which is absent in tea saponin) or to the extra sugar residue in the tea saponin remains unclear for the moment. For both saponins, the hydrophobic outer layer is not densely packed and contains a significant fraction of empty space between the hydrophobic scaffolds. This important result demonstrates that the total saponin adsorption is predominantly governed by the close packing of the hydrated sugar groups which are densely populating the inner layer, adjacent to the water subphase.

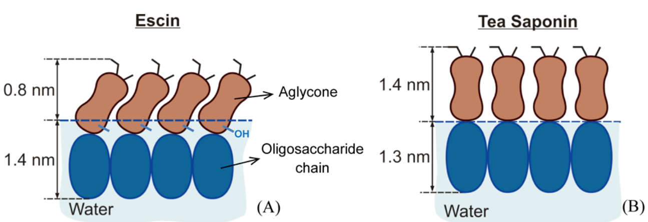

Figure 5. Schematic representation of the structure of the saponin adsorption layer: (A) escin; (B) tea Saponin; consistent with the measured scattering length density profiles.

Surface Tension Data. In addition to the neutron reflectivity measurements, complementary surface tension measurements were made. The surface tension of the saponin solutions were measured by the Wilhelmy plate method, as described earlier. For these solutions, the surface tension decreases with time, and the kinetics of surface tension, $\sigma(t)$, and approach to equilibrium are relatively slow and can take many minutes.[12] Here, an approach which describes $\sigma(t)$ by a biexponential equation with two relaxation times (t_1, t_2)[12] was used

$$\sigma(t) - \sigma_e = \Delta\sigma_1\, e^{-t/t_1} + \Delta\sigma_2\, e^{-t/t_2} \tag{4}$$

where $\sigma(t)$ and σ_e are the current and the equilibrium values of the surface tension, respectively. The time dependence of the surface tension data was described by eq 4. From the best fits, the values of the equilibrium surface tension, σ_e, were determined and used to plot the variation in surface tension with surfactant concentration, as shown in Figure S3 in the Supporting Information. The Gibbs adsorption equation[32,33] was used to obtain an estimate of the adsorption at saturation

$$\frac{d\sigma_e}{d\ln C_S} = -k_B T \Gamma \tag{5}$$

where k_B is Boltzmann constant, T is absolute temperature, C_S is surfactant concentration in the bulk solution, and Γ is surfactant adsorption. The experimental points in the

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concentration range corresponding to $\sigma_e < 65$ mN/m and cmc were interpolated by eq 5 to determine the saponin adsorption, as summarized in Table 3.

Table 3. cmc, Area/Molecule, and Saponin Adsorption at cmc, for Air−Water Interface, as Determined from Surface Tension

saponin type	pH	cmc (mM)	area/molecule (nm^2)	adsorbed amount, Γ ($\mu mol \cdot m^{-2}$)
escin in light water	4	0.06	0.63 ± 0.05	2.6 ± 0.2
escin in light water	natural pH	0.11	0.57 ± 0.06	2.9 ± 0.2
escin in D_2O/H_2O (nrw)	natural pH	0.11	0.54 ± 0.06	3.1 ± 0.2
escin in light water	8	0.072	0.47 ± 0.05	3.2 ± 0.3
tea saponin in light water	natural pH	0.30	0.86 ± 0.08	2.3 ± 0.2
tea saponin in D_2O/H_2O (nrw)	natural pH	0.30	0.66 ± 0.06	2.5 ± 0.2
Supersap in light water[a]	natural pH	0.13	1.1 ± 0.05	1.5 ± 0.05

[a]Data taken from Stanimirova et al.[12]

A comparison of the adsorbed amounts for the escin and tea saponins obtained from neutron reflectivity and surface tension data show some differences. The mean values from neutron reflectivity for the escin and tea saponins are 2.4 and 2.1 μmol·m^{-2}, compared with values from surface tension of 3.0 and 2.4. In both neutron reflectivity and surface tension data for the escin saponin, there is an increase in the adsorption as the pH increases from 4 to 8. From the neutron reflectivity data, it increases from 2.2 to 2.7 μmol·m^{-2}, and from the equivalent surface tension data, the change is from 2.6 to 3.2 μmol·m^{-2}. However, in both cases, the changes are within experimental error. Even at pH 4, where escin is expected to be nonionic, the adsorbed amounts from neutron reflectivity and surface tension are still systematically different. For the Supersap saponin, the adsorbed amount from surface tension is 1.5 μmol·m^{-2} compared with the value of 1.9 μmol·m^{-2} from neutron reflectivity. It has been shown for nonionic surfactants,[34] where generally the adsorption saturates before the cmc, that the adsorption determined from surface tension can be in good agreement with the directly determined surface excesses from neutron reflectivity. In broadest terms, the results from surface tension and neutron reflectivity for escin at different pH, for tea saponin and for Supersap saponin are in agreement when the errors in the measurements are taken into account. Indeed, the general trends, that is, the adsorption is highest for escin, intermediate for tea saponin, and lowest for Supersap saponin, are consistent for both surface tension and neutron reflectivity data.

For ionic or weakly ionic surfactants, Xu et al.[35] have shown that compared with the neutron reflectivity data, the application of eq 5 to surface tension data generally underestimates the saturation adsorption. This is as a result of the variation in the activity in the region of the cmc because of the onset of micellization. Xu et al.[35] showed that this can be accounted for by applying the mass action model of micellization to the surface tension data in that region, where the variation largely depends upon the degree of ionization of the micelles. From the form of the adsorption isotherms measured by neutron reflectivity (see Figures 2 and 3), the saponins are most likely only weakly ionic at any of the solution conditions. However, taking into account such factors would not necessarily account for the differences encountered. For example, surface tension overestimates the adsorption compared with neutron reflectivity for the escin and tea saponins, which is opposite to the trends normally observed.[35] However, for the Supersap saponin surface tension underestimates the adsorption compared with neutron reflectivity. For the saponins, there is another factor, in that it is assumed that the saponins are a single nonionic species, whereas the tea and Supersap saponin extracts contain a range of molecular species with different sugar residues and structures. In both surface tension and neutron reflectivity data analysis, the saponins are assumed to be a single molecular species, as shown in Figure 1. It is possible that the heterogeneity of the materials has a greater impact on the interpretation of the surface tension data than the neutron reflectivity data.

The surface tension measurements for the escin and tea saponins were also made in two different solvent isotopic compositions, in H_2O and nrw (see Table 3 and Figure S3 in the Supporting Information). Generally the evidence in the literature is that in surfactant adsorption there is no measurable isotope effect when H is replaced by D, as demonstrated on a wide range of surfactants,[24] and more recently on some different biosurfactants.[36] Although nrw contains only 8 mol % D_2O the occurrence of strong hydrogen bonding between the saponin sugar groups raises the potential for a deuterium isotope effect, as discussed by Wade.[37] The results summarized in Table 3 show that the differences encountered in H_2O and nrw are within experimental error and that any isotope effect is not significant.

■ SUMMARY AND CONCLUSIONS

The structural measurements with neutron reflectivity show some interesting similarities and differences between the adsorption layers of escin and tea saponins at air−water interface, as illustrated in Figures 4, S2 and 5. The surface structure is dominated by a relatively dense and hydrated headgroup region. The different headgroup structure, with an additional sugar group in the tea saponin, compared with escin, does not result in significant differences in the hydrophilic region. The thicknesses are similar, and the density difference is in part accounted for by the difference in the saturation adsorption for these two saponins. The hydrophobic triterpenoid region is not densely packed and contains no solvent. For escin, this region is rather thin, when compared with the total length of the triterpenoid scaffold, which shows that this hydrophobic region is partially embedded in the aqueous phase and/or tilted with respect to the surface normal. For tea saponin ,this layer is thicker which indicates fully stretched triterpenoid groups, oriented almost perpendicularly to the solution surface. These observations, combined with the fact that both these saponins form highly visco-elastic adsorption layers, support the assumption that strong intermolecular hydrogen bonds occur between the neighboring sugar groups in the adsorption layers, and that these bonds give rise to the observed high surface elasticity.

The molar adsorption decreases with the molecular mass of the saponins and is highest for escin, intermediate for tea saponin, and lowest for *Quillaja* saponin. The total adsorption determined from surface tension is in a broad agreement with

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the results from the neutron reflectivity. However, differences up to 20–30% in the measured adsorption were observed for the results, obtained by the two methods; and such differences are often encountered, as discussed by Li et al.[34] and Xu et al.[35] Here, these differences have a further contributing factor, as saponin extracts studied are typically multicomponent mixtures, containing ionizable carboxylic groups. The simple approach for the interpretation of surface tension data, based on eq 5, may not be appropriate for these systems. Nevertheless, the surface tension data give reasonable estimate of the saponin adsorption and the main trends for the three saponins studied are captured.

The neutron reflection results show that relatively subtle differences in the structure of the adsorbed layers are visible for saponin molecules, without the need for deuterium labeling of the saponin. These structural properties can be potentially correlated with macroscopic surface properties, such as the surface rheology. This approach could be applied to a wider range of similar systems, provided that relatively high purity samples are available.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.8b02158.

Some additional experimental data are available in the Supporting Information (PDF)

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