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Study of Protein–Lipid Interactions at the Bovine Serum Albumin/Oil Interface by Raman Microspectroscopy

GUANGTAO MENG,† JUDY C. K. CHAN,† DÉRICK ROUSSEAU,§ AND EUNICE C. Y. LI-CHAN*,†

Faculty of Agriculture, Food Science Building, The University of British Columbia, 6650 N.W. Marine Drive, Vancouver, British Columbia, Canada V6T 1Z4, and School of Nutrition, Ryerson University, 350 Victoria Street, Toronto, Ontario, Canada M5B 2K3

The interface of 10 or 25% (w/v) bovine serum albumin (BSA), pH 7, buffered solution against mineral or corn oil was studied with a Raman microscope. A gradient of distribution of protein and oil at the interface was observed. The difference spectrum obtained by subtracting the spectrum of mineral or corn oil from that of the BSA/oil interface indicated interactions involving different functional groups of the BSA and the oil molecules. Against mineral oil, the BSA spectrum showed reduced intensity of the tryptophan band at 750 cm^{-1} and reduced intensity ratio of the tyrosine doublet at 850−830 cm^{-1}, indicating changes in the microenvironment of these hydrophobic residues. A negative band at 2850 cm^{-1} indicated the involvement of the CH groups in the mineral oil. However, the amide regions, normally assigned to protein secondary structure, were not significantly changed. When the spectrum of BSA was subtracted from the BSA/mineral oil interface spectrum, the resultant difference spectrum showed changes of symmetric and antisymmetric CCC stretches at 980 and 1071 cm^{-1}, respectively. In contrast, the difference spectrum of BSA/corn oil interface − BSA showed a decrease of CH_2 symmetric stretching at 2850 cm^{-1} and a decrease of unsaturated fatty acid hydrocarbon chain stretch at 3010 cm^{-1}. Raman spectroscopy is a useful tool to study the nature of protein−lipid interactions.

KEYWORDS: Raman microspectroscopy; protein; lipid; interface; bovine serum albumin

INTRODUCTION

A large number of foods exist in the form of emulsions, such as milk, cream, mayonnaise, soups, sauces, and butter. Much research dealing with food emulsion stabilization has examined improvement of kinetic stability and the use of emulsifying agents. Stabilization is usually achieved by adding small surfactant molecules (e.g., polysorbates, phospholipids) and/or proteins. These species populate the oil/water interface and lower the interfacial tension or form a mechanically cohesive interfacial film around the droplets (*1*, *2*).

Proteins, being amphipathic molecules, are often the emulsifier of choice for food processors. From a fundamental perspective, the relationship between protein structure and emulsifying activity depends on a combination of factors such as molecular flexibility, molecular size, surface hydrophobicity, net charge, and amino acid composition (*3*). The structure and physical−chemical properties of lipids also influence the formation and stability of emulsions and texture of many food products (*4*). For example, Funasaki et al. (*5*) discussed the orientation states of triacylglycerols at air/water interfaces and postulated different conformations based on fatty acid chain length. In the case of triacetin, two of the three chains exist in the air and the other is in water, whereas tributyrin, tricaproin, and tristearin have all three of their chains oriented toward the air. All of these properties of either protein or lipid will influence the protein−lipid interactions at the interface and will further affect the formation and stability of the emulsions. An early study (*6*) suggested that protein adsorption to the oil/water interface of food emulsions is diffusion-controlled in the initial stages of protein−interface interactions. However, even now, limited information is available on the exact nature of protein−lipid interactions at the interface.

The surface and interfacial behavior of a number of proteins has been studied, including β-lactoglobulin (*7*), lysozyme (*8*), apomyoglobin (*9*), β-casein (*9*), α-casein (*9*), and BSA (*8*, *9*). In many studies, BSA has often been used as a model globular protein (*9*−*11*). The conformation that a protein adopts at an interface has been analyzed by different techniques, including ellipsometry (*12*, *13*) and fluorescence (*14*, *15*). However, the studies using ellipsometry were mainly focused on quantifying the amount of adsorbed protein, whereas the fluorescence techniques either provided limited information on specific amino acids (i.e., front-face steady-state fluorescence) or lacked a reliable calibration method (i.e., total internal reflectance

* Corresponding author [telephone (604) 822-6182; fax (604) 822-3959; e-mail ecyl@interchange.ubc.ca].
† The University of British Columbia.
§ Ryerson University.

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fluorometry). Spectroscopic methods, including circular dichroism (CD) (*16*), FT-IR (*7*, *17*), and FT-Raman spectroscopy (*4*), have also been applied to study the conformation of proteins in emulsions, foams, and liposomes. However, no direct investigation at the protein/lipid interfaces has been performed.

Raman microspectroscopy is a newly developed technique that has been used in many areas in recent years (*18*−*20*). Equipped with a microscope, the irradiating beam of a Raman microspectroscope may be aimed toward a specific region in the sample with the resulting Raman scattering signals illustrating its spectroscopic behavior. This method has been used in dental research (*21*, *22*) and to characterize the microstructure of foods (*23*). However, no publication has been found on the application of Raman microspectroscopy to the investigation of protein/lipid interfaces in food systems.

In this study, our objectives were to evaluate the potential of using Raman microspectroscopy as a new technique to study the interactions of protein (BSA) and oil (mineral oil or corn oil) at the protein/oil interface.

MATERIALS AND METHODS

Materials. BSA (A-4503, Sigma Chemical Co.; initial fractionation by cold alcohol precipitation, fraction V, minimum 96% by electrophoresis), mineral oil (paraffin oil, light, O121-1, Fisher Scientific), and corn oil (C 8267, Sigma Chemical Co., density = 0.9 g/mL) were purchased from commercial sources and used without further purification.

Methods. The interface of protein and oil was studied with an RM 1000 Raman microscope (Renishaw, Gloucestershire, U.K.). Samples were prepared by layering mineral or corn oil on top of an aqueous solution of BSA (25 or 10% w/v, respectively, in 0.1 M phosphate buffer at pH 7.0) held in a quartz cuvette. Due to the much higher signal/noise ratio obtained for Raman spectra of oil compared to proteins, it was necessary to use the higher protein concentration when the possible changes in secondary structure of BSA at the mineral oil/BSA interface, as reflected in the amide I region, were investigated. After standing for at least 6 h to reach a stable equilibrium of any structural changes upon adsorption of proteins at the interface (*6*), the cuvette was placed on the Renishaw sample platform, which could be moved vertically. A microscope was used to locate the interface between the protein solution and the oil, and the laser beam (782 nm) was then focused on certain positions at the interface. Raman spectra of at least three different positions for each of the interfaces as well as the individual oil and aqueous phases were collected from 400 to 3400 cm^{-1}. The exposure time of the Raman scattering to the CCD camera was set at 60 s, with the cosmic ray removal option on. Three accumulations were collected for each spectrum.

Raman Spectral Data Analysis. The collected Raman spectra were analyzed using Grams 32 (Galactic Industries Corp., Salem, NH) and OMNIC analytical software (Thermo Nicolet Corp., Madison, WI). All spectra were baseline-corrected. All spectra except for those of the oils were smoothed using the Savitsky−Golay nine-point smoothing method. This algorithm, being the most familiar method of smoothing in analytical chemistry, is an indirect filter that fits the spectral data points within a wavenumber interval to a polynomial by the least-squares method. The parameters are the degree of the polynomial and the number of points to fit (*24*). The intensity of protein and interface spectra was normalized according to the protein phenylalanine peak at 1003 ± 1 cm^{-1}. The coefficients of variation for peak intensities of replicate spectra of BSA or mineral oil were <10%.

Difference spectra were calculated by subtracting either the oil or the protein spectrum from the spectrum at the interface. To study possible changes in protein structure at the interface, the subtraction of the mineral oil spectrum from the interface spectrum was performed by zeroing the peak near 2726 cm^{-1}, which was unique to mineral oil and absent in the BSA spectrum. This peak was assigned as the overtone or combination bands of the deformation modes of CH_3 and CH_2 groups, intensity of which is enhanced by interaction with carbon−hydrogen stretching fundamentals of the same symmetry classes (*25*). Similar peaks were found in this region in the standard spectrum for mineral oil reported in the *Handbook of Organic Compounds* (*26*). The resultant difference spectrum (interface − oil) was used for comparison with the original spectrum of BSA to study any changes of the BSA structure in the interface.

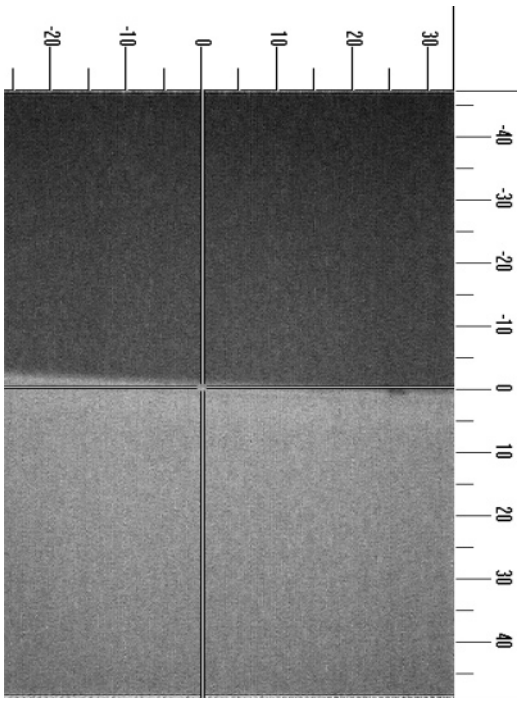

Figure 1. Image of BSA/mineral oil interface under the microscope.

To study the changes of oil structure at the interface, the BSA spectrum was subtracted from the BSA/oil interface spectrum by zeroing the phenylalanine peak of protein near 1003 cm^{-1}, as mineral oil shows no peak in this region. The comparison of the resultant spectrum (interface − BSA) with the mineral oil spectrum was performed by normalizing the intensities according to the 2726 cm^{-1} peak.

The effects of corn oil on the interface were also studied. The spectrum of BSA was subtracted from that of the BSA/corn oil interface by zeroing the 1003 cm^{-1} band. The resultant difference spectrum was compared with the corn oil spectrum by normalizing the intensities according to the 1750 cm^{-1} band. The spectrum of corn oil was subtracted from that of the interface by zeroing the 1750 cm^{-1} band. The resultant difference spectrum was compared with the spectrum of BSA.

RESULTS

Figure 1 shows the image of the interface between 25% BSA and mineral oil, as seen through the microscope of the Raman microspectrometer. The small square at the cross-hair shows the focusing position of the laser, which can be controlled by the software. For example, in **Figure 1**, the cross-hair position was located at the bright line between the oil and protein phases; this position was set as the "0" point. When the cross-hair position was moved from 0 to −10 toward the upper (dark) section of the interface, the laser focus point was shifted 10 μm upward from the zero point at the interface toward the oil phase. Conversely, moving the cross-hair from 0 to +10 toward the lower (lighter) section indicated that the laser focus was shifted 10 μm in the interface toward the aqueous phase. Laser position was monitored for each collected spectrum.

The spectrum of the interface of 25% BSA and mineral oil taken at the "0" point (refer to **Figure 1**) was compared with the individual spectra of 25% BSA and mineral oil (**Figure 2**). Tentative assignments of major spectral bands based on those

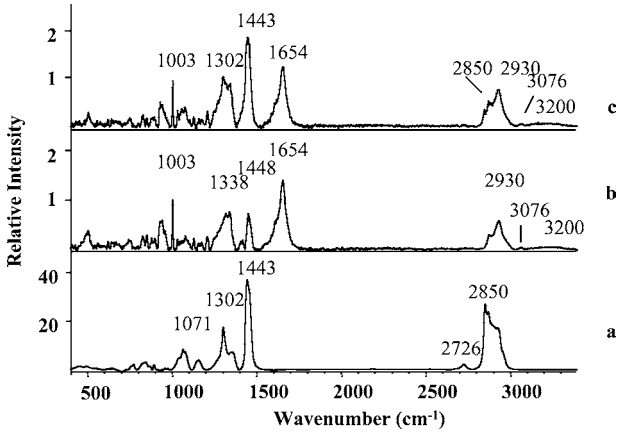

Figure 2. Raman spectra of BSA, mineral oil, and their interface: (**a**) mineral oil; (**b**) 25% BSA; (**c**) BSA/mineral oil interface at the zero point (see **Figure 1**).

Table 1. Assignments of the Major Raman Scattered Bands of Mineral or Corn Oil and BSA Spectrum

wavenumber, cm^{-1}	assignment
mineral oil/corn oil	
800–900	C–C stretching of $-(CH_2)_n-$
1000–1100	C–C stretching of $-(CH_2)_n-$
1302	CH_2 in phase twist
1443	CH_2 scissoring
1660	C═C stretch (*cis*)
1750	C═O ester stretch
2730	deformation of CH_2 and CH_3 groups
2850, 2870	CH_2, CH_3 symmetric stretch
3010	═CH stretch of ═CHR or $=CH_2$ groups
BSA	
503	S–S stretching vibrations (gauche–gauche–gauche)
750	tryptophan
830, 850	tyrosine doublet
936–946	amide III helix
965–970	amide III random
980–990	amide III antiparallel β-sheet
1003	phenylalanine
1338	C–H bending or tryptophan
1448	C–H bending
1654	amide I band
2930	C–H stretching of various amino acids
3076	═C–H stretching of aromatic amino acids
3200	O–H stretching of water molecules

reported in the literature (*4*, *27*−*29*) are shown in **Table 1**. Contributions from both oil and protein components could be seen in the interface spectrum.

Spectra were also collected at different positions in the interface. From the literature, properties of protein and the interface (including the thickness) are affected by many factors including the protein concentration, time to equilibrium, and pressure (*30*). Globular proteins such as BSA tend to migrate toward the interface, forming aggregates and even gel-like structures. A study using a neutron reflectivity method suggested a two-zone structure of BSA stabilized the oil/aqueous interface (*31*). In that study, the BSA monolayer showed a film thickness of ∼2 nm at the outer layer and 1.1 nm at the inner zone. In our case, there is no direct measurement of the thickness of the interface. We consider the interface as the intermediate layer between the positions where only oil or only protein signals were observed in the Raman spectrum. The intensity of peaks assigned to oil decreased when the laser focus was moved within the interface toward the protein phase and vice versa, the intensity of peaks assigned to protein decreased when the laser

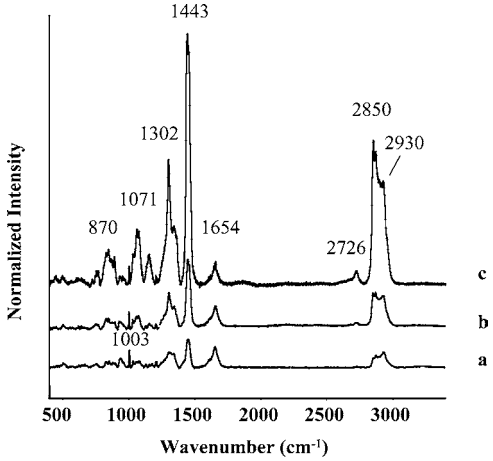

Figure 3. Raman spectra at different positions in the BSA/mineral oil interface: (**a**) 25 μm toward the protein phase; (**b**) at the 0 point; (**c**) 50 μm toward the oil phase. Intensities were normalized according to the 1003 cm^{-1} band.

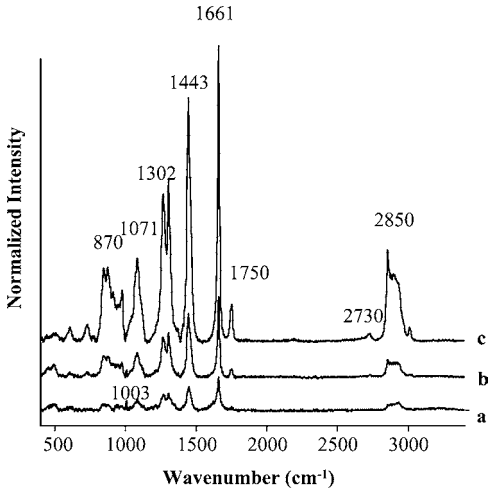

Figure 4. Raman spectra at different positions in the 10% BSA/corn oil interface: (**a**) 500 μm toward the protein phase; (**b**) 300 μm toward the protein phase; (**c**) at the 0 point. The intensities were normalized according to the 1003 cm^{-1} peak.

focus was moved toward the oil phase (**Figure 3c**,**a**, respectively, compared to **Figure 3b**). The results indicated a gradient in the distribution of protein and oil within the interface layer. Similar trends were found in the interfaces between 10% BSA and corn oil (**Figure 4**).

To investigate potential changes in the interface spectrum due to oil−protein interactions, difference spectra were calculated by subtracting either the BSA or the oil individual spectrum from the interface spectrum, which was taken at "0" point. In the absence of interactions, subtraction of the BSA spectrum should yield a difference spectrum (interface − BSA) that is identical to the oil spectrum, whereas subtraction of the oil spectrum should yield a difference spectrum (interface − oil) identical to the BSA spectrum. In fact, the resulting difference spectra (**Figures 5** and **6**) show changes that establish interactions and not simply the coexistence of the protein and lipid components at the interface.

The difference spectrum obtained by subtraction of the mineral oil spectrum is shown in **Figure 5**. Reduction of the normalized intensity of the tryptophan band near 750 cm^{-1}, from 0.184 in the BSA spectrum to 0.153 in the interface − mineral oil difference spectrum, indicates the exposure of tryptophan

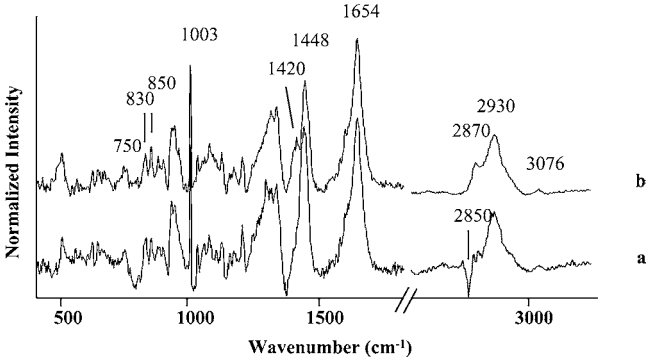

Figure 5. Comparison of the spectrum of BSA with the difference spectrum after subtracting the mineral oil spectrum from the BSA/mineral oil interface spectrum: (**a**) BSA/mineral oil interface − mineral oil; (**b**) 25% BSA. Intensities are normalized according to the 1003 cm^{-1} band.

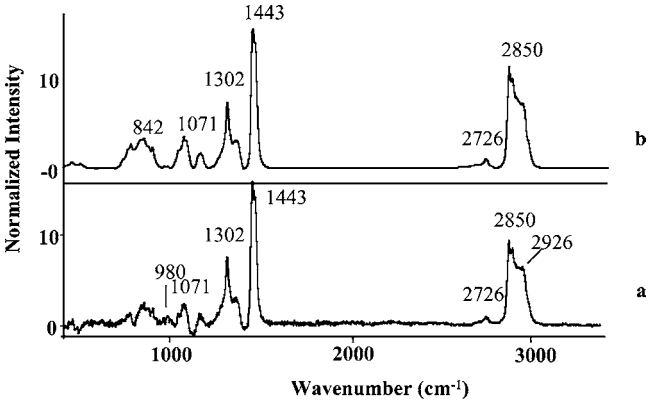

Figure 6. Comparison of the spectrum of mineral oil with the difference spectrum after subtracting the spectrum of BSA from that of the BSA/mineral oil interface: (**a**) BSA/mineral oil interface − BSA; (**b**) mineral oil. Intensities are normalized according to the 2726 cm^{-1} band.

residues, suggesting the denaturation of protein. Exposure of tryptophan due to protein denaturation was also detected in lysozyme−corn oil emulsion systems (*4*). It was suggested that the presence of lipids could alter the molecular structure of proteins and induce the exposure of hydrophobic groups (*4*).

The intensity ratio of the doublet bands at 850 and 830 cm^{-1}, I_{850}/I_{830}, can be used to monitor the microenvironment of the tyrosine side chain (*32*, *33*). In this study, the intensity ratio of the tyrosine doublet was reduced from 1.15 in the BSA spectrum to 0.83 in the interface − mineral oil difference spectrum. The decrease of the I_{850}/I_{830} intensity ratio may be induced by the participation of the tyrosine phenolic groups as hydrogen bond donors (*34*, *35*). The spectra suggested that the oil provides a hydrophobic environment, which was favorable to unfold BSA molecules, causing the exposure of the previously buried residues. The unfolding of the BSA molecule at the interface may in fact expose Trp as well as Tyr residues; however, the interface environment includes both water and oil molecules as the "solvating environment". This agreed with the gradient distribution of BSA and oil at the interface, which was described above. Because the interface probably contains more water molecules than the interior of the protein molecule, the microenvironment of the amino acid residues in the unfolded protein molecule at the interface may be less hydrophobic than the microenvironment of the protein interior. Thus, the Raman spectra suggest that Trp residues of BSA located at the interface are in a less hydrophobic environment than Trp residues of BSA in bulk aqueous solution, where the Trp are likely buried in the protein interior. Although this may also be true for the Tyr residues, the phenolic groups of the Tyr residues in the unfolded BSA may act as strong hydrogen bond donors interacting with water molecules at the interface, and therefore a decrease in the intensity ratio of the tyrosine doublet was observed.

An increase in the normalized intensity of the CH (CH_2 and/or CH_3) bending band close to 1445 cm^{-1} was also observed in the difference spectrum obtained from the subtraction of the mineral oil spectrum. This might be due to the changes of BSA (1448 cm^{-1}, CH bending) or mineral oil (1443 cm^{-1}, CH_2 scissoring), or protein−lipid interactions involving CH groups. The negative signal in the difference spectrum at 2850 cm^{-1} suggested the involvement of CH_2 groups of the mineral oil at the interface.

In contrast to these changes in bands reflecting involvement of Trp, Tyr, and CH (CH_2 and/or CH_3) groups in tertiary structure of BSA at the interface, the interface − mineral oil difference spectrum was similar to the BSA spectrum in the amide I (1654 cm^{-1}) region, suggesting little change of secondary structure at the interface compared to BSA in solution.

To study the behavior of mineral oil in the interface, the BSA spectrum was subtracted from the BSA/mineral oil interface spectrum, and the resultant difference spectrum was compared with the spectrum of mineral oil on its own (**Figure 6**). Both spectra were normalized to the 2726 cm^{-1} peak.

The appearance of a broad band at 980 cm^{-1} in the interface − BSA difference spectrum suggested an increase of symmetric CCC stretch of the mineral oil, whereas a decrease of the 1071 cm^{-1} peak intensity may have been due to a decrease of antisymmetric CCC stretch. The result indicated a conformational change of the mineral oil alkyl chains, which may be caused by the interactions with the protein molecules at the interface.

A decrease was detected in the CH_2 symmetric stretching vibrations of mineral oil, as monitored in terms of intensities of the 2850 and 2870 cm^{-1} peaks, suggesting involvement of CH groups of mineral oil in the interface. The result was in agreement with the negative peak at 2850 cm^{-1} in **Figure 5**, which resulted from subtracting the mineral oil spectrum from that of the interface. Meanwhile, enhanced peak intensity was observed in the interface − BSA difference spectrum at 2926 cm^{-1}, which closely corresponded with the CH stretching scattering (at 2930 cm^{-1}) of the BSA molecule. The result suggested the involvement of CH (CH_2 and/or CH_3) groups of BSA in protein−lipid interactions in the interface.

Corn oil is an edible oil commonly used in foods. Compared with mineral oil, the spectrum of corn oil showed extra bands of C═C stretch at 1660 cm^{-1} and C═O ester stretch at 1750 cm^{-1} (**Figure 7a**). **Figure 7b** shows the difference spectrum of BSA/corn oil interface − BSA. The ester band at 1750 cm^{-1} was used to normalize the spectra as this band was unique in the corn oil spectrum but absent in that of BSA. However, such normalization caused simultaneous and proportional intensity increases of bands at 876, 967, 1075, 1263, 1299, and 1443 cm^{-1}. The phenomena suggested an exaggeration of these bands in the difference spectrum caused by normalization to the 1750 cm^{-1} band. In other words, the intensity of the 1750 cm^{-1} band may in fact have been decreased in the interface spectrum due to the involvement of the C═O ester group of the triacylglycerol in the interaction with protein at the interface. No significant difference was detected for the band intensity at 1660 cm^{-1}. However, a great decrease of the intensity was found at the 2850 (CH_2 symmetric stretching vibration) and 3010 cm^{-1} (unsaturated fatty acid hydrocarbon chain ═C−H) regions. This may suggest a decrease of CH_2 symmetric stretching vibration

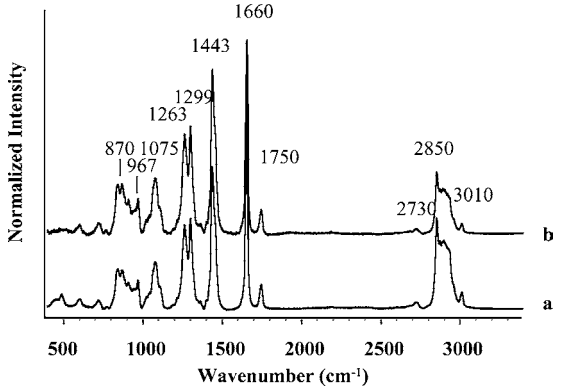

Figure 7. Comparison of the spectrum of corn oil with the difference spectrum after subtracting the spectrum of BSA from that of the BSA/corn oil interface: (**a**) corn oil; (**b**) BSA/corn oil – BSA. Intensities are normalized to the 1750 cm^{-1} band.

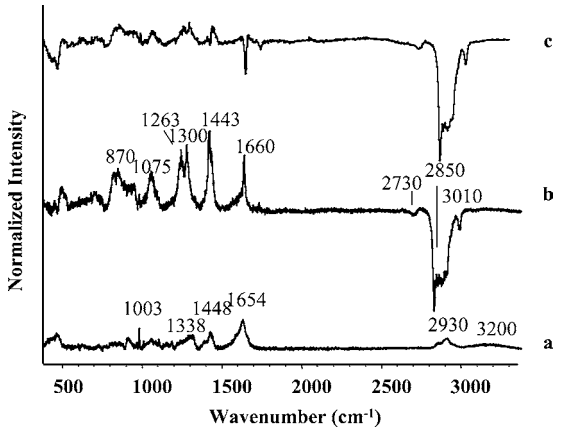

Figure 8. Comparison of the spectrum of BSA with the difference spectrum after subtracting the corn oil spectrum from that of the BSA/corn oil interface: (**a**) 10% BSA; (**b**) interface – corn oil; (**c**) further subtraction of **b**. Intensities are normalized according to the 1003 cm^{-1} band.

of corn oil at the interface, indicating involvement of CH groups of corn oil in the protein−lipid interactions. As mentioned above in the case of mineral oil, in addition to the decrease of the CH_2 symmetric stretching vibrations at 2850 cm^{-1}, the CCC stretch of mineral oil was changed from antisymmetric to symmetric due to the interaction with protein at the interface. However, in the case of the corn oil, it was the CH_2, =C−H groups and ester groups of the triacylglycerol that were involved in the interactions between the protein and corn oil in the interface. The triacylglycerol structure of corn oil may confer a relatively stable CCC backbone conformation of corn oil when compared with mineral oil.

The spectrum of corn oil was also subtracted from that of the interface, and the difference spectrum was compared with the spectrum of BSA (**Figure 8**). The difference spectrum (**Figure 8b**) showed positive peaks at 800−900, 1075, 1263, 1300, 1443, and 1660 cm^{-1}. Those peaks were found in the corn oil spectrum (**Figure 7a**), suggesting an insufficient subtraction of the corn oil spectrum from that of the interface, perhaps due to a decreased intensity in the interface spectrum of the 1750 cm^{-1} band, which was used as the standard band for subtraction of corn oil. This agreed with the result shown in **Figure 7**. Further subtraction (**Figure 8c**) was performed to level off these extra oil peaks in the difference spectrum. Both of the 1660 and 1750 cm^{-1} peaks were negative in the resulting difference spectrum (**Figure 8c**), reflecting the involvement of C=C stretching and changes in the COO(R) groups from the corn oil triacylglycerol molecules as a result of the interaction with BSA. Negative peaks in the C−H stretching region from 2700 to 3100 cm^{-1} also suggested that corn oil molecules were not simply coexisting with protein solution but that interactions occurred between the two phases. The CH_2, CH_3, and =C−H bonds of corn oil were involved in the interaction, which resulted in the appearance of the negative peaks. The result is in agreement with that of the difference spectrum of BSA/corn oil interface − BSA (**Figure 7**). Conclusions on the effect of corn oil on BSA structure could not be made, due to the weak signals in the difference spectrum at the protein concentration (10%) used for the BSA/corn oil interface (**Figure 8**) compared to the BSA/mineral oil interface (**Figure 5**). Changes in BSA could also not be observed when 10% BSA/mineral interfaces were studied or by focusing the laser at different regions of the interface closer to the protein phase (data not shown).

DISCUSSION

The properties and behavior of many biomaterials including foods depend on their microstructure. Examples of these properties are the scoopability of ice cream, the spreadability of margarine/butter, the pourability of ketchup, flavor release (property similar to drug delivery), etc. Many food researchers have tried to relate food property and structure by studying their rheological behavior and microscopic structure (*36*, *37*). However, there is still a "what and where" question, that is, in our case, we need to know what kind of interactions are happening at the interface and where they are.

Raman microspectroscopy has the possibility to tackle this problem. Using the confocal mode of this method, the subcellular structures in both animals (*18*) and plants (*19*) have been successfully mapped. It is also widely used in material polymer science to map the relative spatial location and concentration of polymers (*20*).

In the current study, with the help of the microscope, the laser can be focused at any position in the sample and the image of the studied field can be carefully monitored. The Raman spectrum and the image taken can therefore be closely related to each other. The laser used in this study was located by the side of the sample platform, which allows the beam to be directed horizontally through the interface. The confocal mode was not necessary in this case, as the interface was expected to be homogeneous in this direction. To study changes in the protein and oil components at the interface arising from the interactions between these constituents, calculation of difference spectra may be a useful approach to observe the spectral contribution of protein only or oil only.

The results of this study indicated conformational changes of BSA at the BSA/mineral oil interface. In comparison to the overlapping of protein and oil peaks in the case of the BSA/corn oil interface, especially in the amide I region, the relatively simple Raman spectrum of mineral oil was particularly useful to facilitate the investigation of BSA structural changes at the interface. The extent of protein structural changes at the air/water or oil/water interfaces depends on the conformational stability of the protein involved, as well as the temperature, pH, ionic strength, etc. (*38*). Most proteins, especially globular proteins, have an intricate secondary and tertiary structure. As a globular protein, BSA has a folded structure in aqueous solution that is stabilized by 17 intramolecular disulfide bonds (*9*) and may exist in a compact form surrounded by a hydration shell. In this study, the unfolding of BSA molecules at the BSA/mineral oil interfaces appeared to be limited to changes

occurring at the tertiary structural level. Little or no change in the secondary structure was observed. This finding is consistent with results reported in a study performed by using an electroacoustic method on BSA-stabilized soybean oil-in-water emulsions, which also showed limited conformational changes of BSA at the interface (*39*). In contrast, flexible proteins such as casein can adopt a number of different conformational states when they are adsorbed at an oil-in-water interface (*40*−*43*). Circular dichroism and infrared spectroscopic studies of adsorbed protein (BSA and β-lactoglobulin) also showed the tertiary structure to be more drastically affected by adsorption than was the secondary structure (*16*). Similar phenomena were observed by Fang and Dalgleigh (*7*) while studying β-lactoglobulin absorbed to the interface of soybean oil and water by FTIR. These authors suggested that, given the amphipathic nature of α-helices (residues 130−140 of β-lactoglobulin), the protein molecules may have participated in adsorption without significant changes in structure.

Given that the exposure of hydrophobic regions in a protein to an aqueous environment is considered to be energetically unfavorable, hydrophobic interactions likely played a key role in the adsorption behavior of BSA at the mineral oil interface. Walstra and de Roos (*2*) suggested that hydrophobic interfaces (air/water and oil/water) permitted conformational changes during protein adsorption, mentioning that the fluid interface allowed for conformational changes in the bulk phase and protrusion of hydrophobic regions into the nonaqueous phase. The hydrogen bonding between Tyr residues of BSA and water molecules may also contribute to the interactions at the interface. Electrostatic interactions sometimes also play a part in the protein−lipid interactions. For example, the association of cytochrome *c* has been considered by many authors (*44*−*46*) as a paradigm of electrostatic binding of peripheral protein to lipid bilayer due to the long-range electrostatic forces in membrane mobility. However, the electrostatic interactions did not show much effect at the BSA/mineral oil interface, because the mineral oil contains mainly *n*-alkanes, which have no charged groups that might play a part in electrostatic interactions.

The difference spectra indicated that both the protein and lipid molecules underwent structural changes at the interface, perhaps indicating interaction between the species or, at the very least, a reorganization as a result of the presence of the interface. Walstra and de Roos (*2*) found that proteins behaved differently when absorbed at triglyceride oil/water, paraffin oil/water, and air/water interfaces. It is also known that triglycerides (e.g., corn oil) used in foods have lower interfacial tension at the oil/water interface than pure hydrocarbons (e.g., mineral oil) (*47*). However, little research has been performed on conformational changes at the protein/lipid interface of edible oils. Dimitrova et al. (*10*) suggested that the protein−lipid interactions in membranes are caused by weak associations of the two molecules, which may influence the membrane lipid structure depending on the type of protein and its ability to adsorb on the lipid membrane. The current study shows the conformational changes of the hydrocarbon backbone in terms of CCC symmetric (e.g., increased intensity of 980 cm^{-1} band) and antisymmetric structure (e.g., decrease of 1071 cm^{-1} band) of mineral oil in the interface. In comparison, in the case of the corn oil interface, it was mainly the CH_2 symmetric stretching modes (e.g., changes at 2850 cm^{-1}) and the ester group (e.g., changes in 1750 cm^{-1}) in the triacylglycerol structure that were involved in the interaction with BSA.

In summary, the protein−lipid interaction in the BSA/mineral oil interface caused structural changes of both protein and oil molecules. Protein denaturation was induced due to the presence of oil, causing the unfolding of protein molecules. The results indicated that the conformational changes of BSA molecule involved the tertiary structure rather than the secondary structure. Corn oil and mineral oil behaved differently at the interface. The involvement of corn oil in the interface was through CH_2, CH_3, COOR, and =C−H groups. However, at the interface of BSA/mineral oil, the CCC stretch of the hydrocarbon backbone chains of mineral oil molecules were also affected by the protein−oil interaction. The hydrophobic interactions involved both the protein and lipid components. Hydrogen bonding also played a role in the protein−lipid interaction at the interface.

This study demonstrated that Raman microspectroscopy can provide a useful tool for studying protein−lipid interactions at the interface. Compared with traditional spectroscopic methods, Raman microspectroscopy has the advantage of being able to monitor structural changes at the location of interest. Application of the approach described in this study may be used to investigate the behavior of proteins at interfaces with various oils under different environments such as pH or the presence of other food components or as a function of processing conditions. Nevertheless, it should be noted that the weak Raman scattering signals from protein molecules necessitate that the protein samples are at relatively high solute concentrations. Furthermore, because the spectral changes that result from interactions at the interface may be subtle, application of more sophisticated data processing techniques such as partial least-squares regression analysis (*48*) may be required to provide a more quantitative analysis of the structural changes and to predict their potential significance in functionality of the protein/lipid system.

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