Study of Binding of 12(*S*)-Hydroxy-5(*Z*),8(*Z*),10(*E*),14(*Z*)-eicosatetraenoic Acid to Bovine Serum Albumin Using Dynamic Surface Tension Measurements

P. CHEN,[†] Z. POLICOVA,[‡] C. R. PACE-ASCIAK,[§] AND A. W. NEUMANN*,[‡]

Contribution from *Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1, Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, Ontario, Canada M5S 3G8, Division of Neurosciences, Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8, and Departments of Pharmacology and Pediatrics, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8.*

Received April 19, 1999. Accepted for publication September 3, 1999.

Abstract □ In a recent paper,[1] we demonstrated that molecular interactions between biopolymers and other smaller molecules can be detected by means of dynamic surface tension measurements. In the present paper, we demonstrate that the same methodology can be employed for investigating dose effects and specificity of molecular interactions. Three similar lipids were chosen for this study: 12(*S*)-hydroxy-5(*Z*),8(*Z*),10(*E*),14(*Z*)-eicosatetraenoic acid (12(*S*)-HETE-free acid), methyl 12(*S*)-hydroxy-5(*Z*),8(*Z*),10(*E*),14(*Z*)-eicosatetraenoate (12(*S*)-HETE-methyl ester), and 5(*Z*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic acid (arachidonic acid-free acid). These substances were added to a fatty acid free bovine serum albumin (BSA) aqueous solution at different lipid concentrations. The characteristic tension response indicates that molecular interactions between 12(*S*)-HETE-free acid and BSA exist. The detected interactions are concentration dependent: at a molecular ratio of lipid to protein of 1:1, the binding of 12(*S*)-HETE-free acid to BSA is hydrophobic in nature; at the molecular ratio of lipid to protein of 10:1, a secondary binding occurs and is hydrophilic in nature. Similar molecular interactions were not detected between 12(*S*)-HETE-methyl ester or arachidonic acid-free acid and BSA, indicating that the interactions between 12(*S*)-HETE-free acid and BSA are specific. As an independent means, surface elasticity is used to probe the molecular interactions at the interface. In the case of 12(*S*)-HETE-free acid but not its methyl ester or arachidonic acid, distinct higher surface elasticities were observed at lipid concentrations in excess of a molecular ratio of lipid to protein of 1:1. This finding reinforces the above stipulations.

1. Introduction

Intermolecular interactions between macromolecules and smaller organic molecules at different surfaces are of fundamental importance to various industrial and biological processes. Mixtures of polymers and surfactants and their surface adsorption kinetics often play a vital role in emulsions or dispersions of a large number of chemical engineering products.[2−5] The interplay between proteins and lipids or lipidlike biomolecules is central to one of the most important functions of proteins, namely the adsorption at biological interfaces, and the structure of biomembranes.[6−8] Although a large amount of work has been done in this area,[5,9−12] the fundamental understanding of the mechanisms is limited. This is partially because most studies have focused on the isotherm, i.e., the equilibrium behavior of polymers or proteins and smaller molecules at interfaces. The dynamic processes, which are far more important in many biological and engineering systems, have been largely ignored.

Recently, we have developed a dynamic surface tension method for studying the dynamics of protein−lipid interactions at interfaces.[1] This method depends on the measurement of dynamic surface tension response to a saw-tooth change in surface area, where both protein and lipid are competing to adsorb. By analyzing the tension response, one can obtain information about both surface competitive adsorption and molecular interactions between the two molecules. Using this method, we studied the binding of Hepoxilin, a newly developed lipidic biomolecule derived from arachidonic acid, to bovine serum albumin.[1]

The dynamic surface tension has been measured by axisymmetric drop shape analysis (ADSA).[13−16] This approach to obtaining surface tension is based on the shape of a sessile or pendant drop. In essence, the shape of a drop is determined by a combination of surface tension and gravity effects. Surface forces tend to make drops spherical whereas gravity tends to elongate a pendant drop or flatten a sessile drop. When gravitational and surface tensional effects are comparable, then, in principle, one can determine the surface tension from an analysis of the shape of the drop. ADSA is devised specifically for drops with axial symmetry. Over the last 15 years, this technique has been developed to allow for measurement of a wide range of surface tensions under both static and dynamic conditions.[13−16]

The advantages of ADSA are numerous as compared with conventional surface tension techniques such as the Wilhelmy plate, the du Noüy ring tensiometer, and those based on the volume or weight of a pendant drop.[8,16] For example, in comparison with the Wilhelmy plate technique, ADSA requires only small amounts of the sample liquid; this becomes significantly advantageous when the sample is rare and expensive to obtain, such as in the case of many biological studies. ADSA also easily facilitates the study of both liquid−vapor and liquid−liquid interfacial tensions; it has been applied to materials ranging from organic liquids to molten metals, and from pure solvents to concentrated solutions. Measurements with ADSA have been satisfactorily made over a wide range of temperature and pressure.[13−16] In addition, since the profile of liquid drops may be recorded by photographs or digital image representation, it is possible to study surface tension in dynamic systems where the properties are time-dependent.

* To whom correspondence should be addressed.
[†] Department of Chemical Engineering, University of Waterloo.
[‡] Department of Mechanical and Industrial Engineering, University of Toronto.
[§] The Hospital for Sick Children and Departments of Pharmacology and Pediatrics, University of Toronto.

10.1021/js9901256 CCC: $18.00
Published on Web 10/30/1999

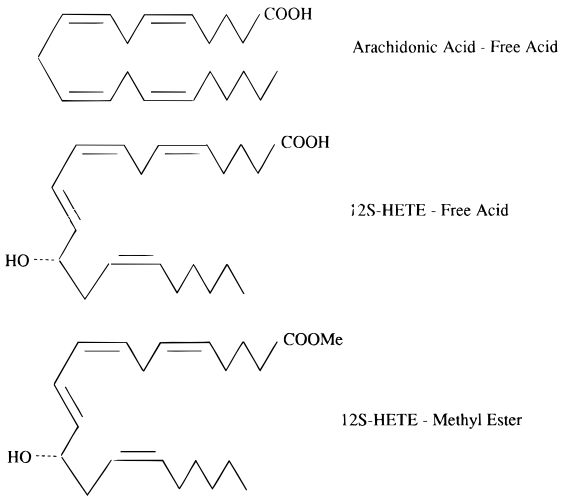

Figure 1—Schematic of molecular structures for (a) 12(*S*)-hydroxy-5(*Z*),-8(*Z*),10(*E*),14(*Z*)-eicosatetraenoic acid (12(*S*)-HETE-free acid), (b) methyl 12(*S*)-hydroxy-5(*Z*),8(*Z*),10(*E*),14(*Z*)-eicosatetraenoate (12(*S*)-HETE-methyl ester form), and (c) 5(*Z*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic acid (arachidonic acid-free acid). It is seen that 12(*S*)-HETE-free acid differs from arachidonic acid-free acid by having an additional hydroxyl group and a cis–trans conjugated diene system, while the difference between 12(*S*)-HETE-free acid and 12(*S*)-HETE-methyl ester is at the carboxyl end.

A number of studies have been carried out on biological interfaces using ADSA.[17−20] For example, the interfacial tension, and hence the surface energetics, of human serum albumin at the water−decane interface was studied as a function of temperature and protein concentration.[17,18] The results allow one to infer protein affinity for such an interface as well as a surface charge distribution of the protein molecules. Incorporating a captive bubble method and ADSA, one can also study low surface tension phenomena in lung surfactant systems and hence draw physiological significance of these substances.[21,22] The study of intermolecular interactions between proteins and lipids has been a recent application of ADSA dynamic surface tension measurements.[1]

In most biological systems, two questions are usually asked in connection with molecular interactions such as binding: one, is there a dose effect, i.e., is there a concentration dependence of the binding; the other is the specificity, i.e., is such binding specific to the molecules under study. In this paper, we demonstrate that the dynamic surface tension method[1] can be employed for the study of dose effects and specificity of molecular interactions. We investigate the molecular interactions between delipidated bovine serum albumin (BSA) and three slightly different lipids: 12(*S*)-HETE-free acid, 12(*S*)-HETE-methyl ester form, and arachidonic acid-free acid. Figure 1 shows their molecular structures; it can be seen that 12(*S*)-HETE-free acid differs from arachidonic acid by having an additional hydroxyl group and a cis−trans conjugated diene system, while the difference between 12(*S*)-HETE-free acid and 12(*S*)-HETE-methyl ester is at the carboxyl end. It was interesting to investigate whether these subtle structural differences would distinguish one molecule from another in terms of their interactions with bovine serum albumin at the surface.

Biologically, the study of the molecular interactions between lipids and protein also has important practical implications. The binding of lipid to a membrane protein would significantly alter the surface properties of the membrane, such as hydrophobicity, and hence affect membrane-assisted enzymatic reactions involving, for example, phospholipases,[23,24] i.e., possibly ion channel behavior and adhesion of cells. Indeed 12(*S*)-HETE has been shown to affect phospholipases[23,24] and potently causes tumor cells to adhere to the vascular endothelium.[25]

The objectives of this paper are (1) to measure dynamic surface tension response to a saw-tooth change in the surface area of solution drops using axisymmetric drop shape analysis (ADSA); (2) to probe the molecular interactions or binding between BSA, as a model protein, and three lipids; (3) to investigate the concentration dependence and specificity of the molecular binding; (4) to present a new means to characterize surface molecular interactions by calculating surface elasticity. The last objective is not only an additional means to detect molecular interactions but also provides a further physical/mechanical property of the lipid−protein monolayer.

2. Materials and Methods

A. Materials—The sample of bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was essentially fatty acid and globulin free, with an average molecular weight of 67 000. It was used without further purification. Deionized and glass-distilled water was used. 12(*S*)-HETE-free acid and arachidonic acid were purchased from Cayman Chemicals (Ann Arbor, MI). The methyl ester derivative was prepared with an ether solution of diazomethane.[26] The reaction for methyl ester synthesis was complete as judged by the layer chromatography. The material was purchased from Cayman Chemicals as the free acid and converted into the methyl ester by methods used routinely in our laboratory, i.e., ethereal diazomethane. The molecular weights of these compounds are 320, 304, and 334, respectively. Since these lipids are not soluble in water, they have been initially dissolved in 1 μL of dimethyl sulfoxide (DMSO) before addition to 1 mL BSA aqueous solutions. Three types of mixed solutions were prepared: (1) 0.02 mg/mL BSA aqueous solution containing 12(*S*)-HETE-free acid at a concentration ranging from 0.001 to 1.0 μg/mL. Note that, within this range, a concentration of 0.1 μg/mL corresponded to a molecular ratio between 12-HETE-free acid and BSA of approximately 1:1; (2) 0.02 mg/mL BSA aqueous solution containing 12(*S*)-HETE-methyl ester at a concentration ranging from 0.01 to 1.0 μg/mL; (3) 0.02 mg/mL BSA aqueous solution containing arachidonic acid at a concentration ranging from 0.001 to 1.0 μg/mL. Two control experiments were performed using the following two solutions: (1) a pure BSA aqueous solution at a concentration of 0.02 mg/mL and 1 μL of DMSO; (2) a BSA-free solution of 1 μg of 12(*S*)-HETE-free acid in a mixture of 1 μL of DMSO in 1 mL of water (instead of a BSA solution).

B. Axisymmetric Drop Shape Analysis-Profile (ADSA-P)—The dynamic surface tension response to a saw-tooth variation in surface area was measured by ADSA-P. Detailed descriptions of ADSA-P are given elsewhere.[13−20] Briefly, ADSA-P fits the theoretical drop profile dictated by the Laplace equation of capillarity to the experimentally determined drop profile. An objective function is constructed which describes the deviation of the theoretical profile from the experimental one. This function is minimized by a nonlinear least-squares regression procedure, yielding the interfacial tension. The program also provides the drop volume, surface area, and the radius of curvature at the apex. The program requires several randomly chosen coordinate points along the drop profile, the value of the density difference across the interface, and the magnitude of the local gravitational constant as input. Each single image of a drop is analyzed 10 times with 20 different, arbitrary profile coordinate points each time.

During the experiment, the sample solution formed a pendant drop at the tip of a Teflon capillary, enclosed in a quartz cuvette which was mounted in an environmental chamber. The surface area of the drop was varied in a saw-tooth pattern through the volume change produced by a motorized syringe connected to the other end of the Teflon capillary.[1] In each run, drop images were captured at 0.3 to 1 s intervals for 6 min. Within this time period, repeated tension response cycles were established, based on which the analyses for molecular interactions were conducted. All the experiments were performed at 37 °C.

3. Results and Discussion

A. Molecular Interactions and Dose−Effects—Figure 2 shows the dynamic surface tension response to

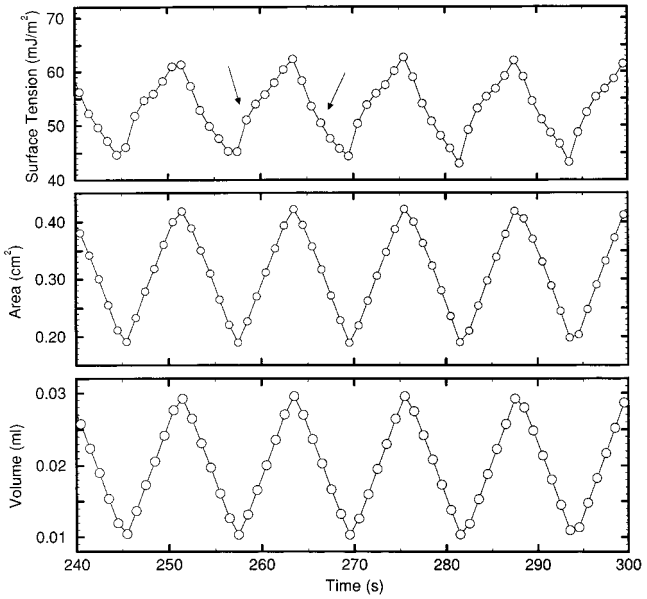

Figure 2—Dynamic surface tension response to a saw-tooth change in surface area, within the time range of 240 to 300 s from the beginning of the experiment with the BSA aqueous solution at a concentration of 0.02 mg/mL and 1 μL DMSO. Each cycle shows a characteristic, skewed shape in the tension response, with two kinks in the two branches corresponding to surface expansion and compression, respectively.

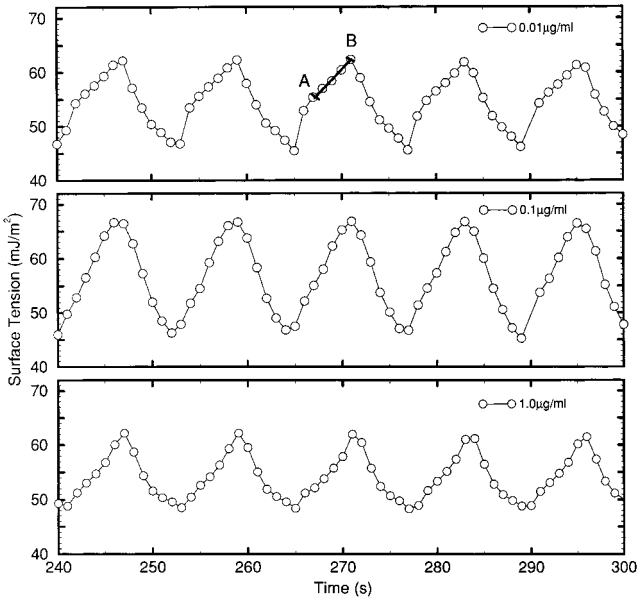

Figure 3—Dynamic surface tension response to the same saw-tooth variation in surface area as that in Figure 2 for the BSA solution to which 12(*S*)-HETE-free acid had been added. A series of 12(*S*)-HETE-free acid concentrations were measured: 0.001, 0.005, 0.01, 0.02, 0.05, 0.1, and 1.0 μg/mL of BSA aqueous solution; however, only three concentrations are shown here since for concentrations below 0.1 μg/mL the tension response is similar to that of 0.01 μg/mL. It is seen that at low 12(*S*)-HETE-free acid concentrations the tension response to the area variation is similar to that observed in Figure 2. However, at the concentration of 0.1 μg/mL, a distinct pattern change is observed in the dynamic surface tension response: the skewed pattern in the tension response of the BSA solution is replaced by a rather symmetric one. As the concentration of 12(*S*)-HETE-free acid increases to 1.0 μg/mL, yet another different, symmetric pattern is observed in the tension response, indicating a dose effect on the surface tension behavior and hence on the surface physicochemical properties.

the saw-tooth change in surface area within the time range from 240 to 300 s from the beginning of the experiment with the aqueous BSA solution at a concentration of 0.02 mg/mL. It has been shown[1] that at early times (less than 60 s) the tension response reflects the initial adsorption process of BSA to the surface and does not repeat itself from cycle to cycle. Only after 120 s does the tension response start showing constant cycles, as shown in Figure 2. Each cycle shows a characteristic, skewed shape, with two kinks occurring in the two branches corresponding to surface expansion and compression.

As demonstrated in a previous paper,[1] the error associated with each individual surface tension value is generally small, less than 0.2 mJ/m^2 at the 95% confidence level; hence, the tension response to the saw-tooth variation in surface area (Figure 2) reliably represents the true physicochemical properties of the surface of a protein and/or lipid adsorption film.

Figure 3 shows the dynamic surface tension response to the same saw-tooth variation in surface area as that in Figure 2 for the BSA solution to which 12(*S*)-HETE-free acid had been added. A series of 12(*S*)-HETE-free acid concentrations were used in the experiment: 0.001, 0.005, 0.01, 0.02, 0.05, 0.1, and 1.0 μg/mL BSA aqueous solution; however, only three concentrations are shown in Figure 3 since for concentrations below 0.1 μg/mL the tension response was found to be similar to that of the concentration of 0.01 μg/mL. It can be seen that at low 12(*S*)-HETE-free acid concentrations ($\leq$0.05 μg/mL) the tension response to the area variation is similar to that observed in Figure 2 for the pure BSA solution: the characteristic, skewed shape indicates that the surface is covered mainly with BSA molecules. However, at the concentration of 0.1 μg/mL, a distinct pattern change is observed in the dynamic surface tension response: the skewed pattern of the BSA solution is replaced by a rather symmetric one. This indicates that the surface properties are not determined solely by BSA, i.e., the added 12(*S*)-HETE-free acid plays a role. It is noted that the 0.1 μg/mL concentration corresponds to a molecular ratio between 12(*S*)-HETE-free acid and BSA of approximately 1:1. As the concentration of 12(*S*)-HETE-free acid increases to 1.0 μg/mL, yet another asymmetric pattern emerges in the tension response. Clearly, there is a dose–effect on the surface tension behavior and hence on the physicochemical properties. At concentrations above 0.1 μg/mL, the tension response to the area change provides a useful tool to probe possible molecular interactions between 12(*S*)-HETE-free acid and BSA.

To investigate further the effects of 12(*S*)-HETE-free acid, a control experiment was performed, in which 1 μg of 12(*S*)-HETE-free acid dissolved in 1 μL of DMSO was added to 1.0 mL water, i.e., in the absence of BSA. The results are shown in Figure 4, where minimal changes in dynamic surface tension pattern are observed in response to the same saw-tooth variation in surface area as that in Figures 2 and 3. If there were no interaction between 12-(*S*)-HETE-free acid and BSA in the mixed solution, the resulting surface tension would have to be a superposition of the surface tensions of the individual lipid and protein solution. However, from Figures 2–4, the tension response of the mixture at a concentration of 12(*S*)-HETE-free acid of 0.1 μg/mL does not reflect the pattern of the pure BSA solution at all. Therefore, molecular interactions must exist between 12(*S*)-HETE-free acid and BSA, likely forming lipid–protein complexes due to 12(*S*)-HETE-free acid interacting with BSA. These complexes, being species different from albumin alone, no longer show the skewed shape of BSA, presumably caused by conformational changes.

In general, the appearance of kinks in the tension response to surface area variations (e.g., Figure 2) reflects a phase or structural transition of the surface molecules.[8] Disappearance of the kinks, at the lipid concentration of

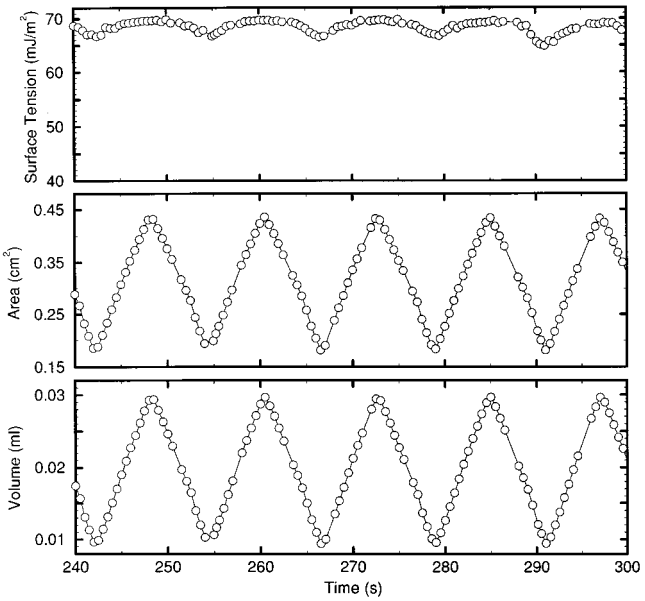

Figure 4—Dynamic surface tension response to the same area variation as that in Figure 2 for a control experiment in which 1 μg of 12(*S*)-HETE-free acid dissolved in 1 μL of DMSO was added to 1.0 mL of water, in the absence of BSA. Rather symmetric cycles with small amplitudes are observed for the tension response.

0.1 μg/mL (Figure 3), indicates that the binding of lipid to protein stablizes the surface phase at one molecular configuration. Furthermore, since the distinct change in the tension response pattern of BSA occurs at the concentration of 0.1 μg/mL of 12(*S*)-HETE-free acid, corresponding to a molecular ratio of 1:1, such interactions between 12-(*S*)-HETE-free acid and BSA are presumably connected with a single binding site (see also below).

Another observation in Figure 3 is that the tension value reached at the peaks for the concentration 0.1 μg/mL is significantly higher than that for the other concentrations including the pure BSA solution (Figure 2). This indicates that the mixture, at the 1:1 molecular ratio, is more hydrophilic than BSA itself; therefore, the molecular interactions between 12(*S*)-HETE-free acid and BSA may be hydrophobic in nature, such that the hydrophobic end of the lipid attaches to a similar part of the protein, leaving the hydrophilic end of the lipid exposed to the surrounding water environment.

At the 12(*S*)-HETE-free acid concentration of 1.0 μg/mL (Figure 3), the molecular ratio between lipid and protein is roughly 10:1. One might think that the resulting dynamic surface tension should predominantly be due to the presence of 12(*S*)-HETE-free acid at the surface. However, the tension response of the lipid alone (Figure 4) shows a very different response, with a much smaller amplitude. If we were to assume that BSA has only one binding site for 12(*S*)-HETE-free acid, then 9 out of 10 lipid molecules would exist in water freely, and the resulting surface properties of the mixture would have to be dominated by the surface properties of the free lipid. However, from Figure 4, the free lipid solution has high surface tension values, above 65 mJ/m^2; from Figure 3, the maximum tension value is also above 65 mJ/m^2 for the mixture of the lipid and the protein at the concentration of 0.1 μg/mL. If there were no interaction between lipid and protein−lipid complex, the tension response of the combination would be expected to be at the same high level. This, however, is not the case; the maxima for 1.0 μg/mL in Figure 3 are clearly below 65 mJ/m^2. Therefore, one needs to conclude that not all additional lipid molecules remain free, but rather bind to BSA, at least to such a degree that the surface tension is significantly lowered. Since the maximum tension value at the 1.0 μg/mL concentration is smaller than that at the 0.1 μg/mL concentration, the new complex, as a result of the new binding of the lipid to the protein, is more hydrophobic, compared with the protein−lipid complex of 1:1 ratio, i.e., 0.1 μg/mL of 12(*S*)-HETE. Hence, the additional binding of the lipid to the protein is presumably hydrophilic in nature.

It should be noted that, even at 12(*S*)-HETE-free acid concentrations lower than 0.1 μg/mL, one may not rule out the possibility of the aforementioned new binding of lipid to protein, or secondary binding of lipid to protein. Such binding is presumably associated with some low affinity sites, and hence it does not amount to any sizable extent and contributes little to the tension response. At 1:1 molar ratio (corresponding to 0.1 μg/mL) or less, 12(*S*)-HETE-free acid mainly interacts with BSA at the single binding site of high affinity; this will predominantly dictate the properties of the surface molecules and affect the pattern of the tension response. Only at higher molar ratios, e.g., 10:1, as the high affinity binding site has already been occupied, this secondary binding becomes important in changing the tension response (Figure 3).

In the above analysis leading to the conclusion of 12(*S*)-HETE-free acid binding to BSA and the dose-dependence of such molecular binding, we tacitly assumed that the DMSO, used as dissolving agent for the lipid, does not interact with BSA, nor does the DMSO play a role in the surface tension response after the initial few cycles. It has been established that, for a mixture of DMSO and BSA, the surface molecular population is dominated by small DMSO molecules only at early stages of the cycling experiment below 60 s, due to DMSO's much higher diffusion coefficient.[1] With the passage of time, BSA gradually adsorbs at the surface, and BSA molecules stay at the surface once they adsorb. This leads to a squeeze-out of the DMSO molecules from the surface. After repeated cycles, the surface properties are essentially determined by the BSA molecules adsorbed at the surface, and no DMSO contribution to the surface tension response can be detected. This indicates that DMSO is merely a vehicle for carrying lipids, and it does not contribute to the tension response at late stages of the cycling experiment, as shown in Figures 2−6.

B. Specificity—To study the possible specificity of the molecular binding of 12(*S*)-HETE-free acid to BSA, two similar lipids, 12(*S*)-HETE-methyl ester and arachidonic acid-free acid, were used to perform the same tension response experiment as that for 12(*S*)-HETE-free acid. The results for 12(*S*)-HETE-methyl ester are shown in Figure 5 for three concentrations at 0.01, 0.1, and 1.0 μg/mL. Again, 0.1 μg/mL corresponds to a molecular ratio of lipid to protein of approximately 1:1. All three concentrations show a skewed pattern, similar to that of the pure BSA solution (Figure 2). This suggests the dominance of protein adsorption at the surface, while 12(*S*)-HETE-methyl ester molecules play little role in producing the surface tension response. When comparing Figure 5 with Figure 3 at the same concentrations, the disappearance of kinks in the tension response differentiates the two lipids in their interations with BSA.

It has been established that there are generally one to two high affinity fatty acid-binding sites on albumin and a number of low affinity sites.[27−29] The distinct difference between Figure 5 and Figure 3 indicates that the lipid−protein interactions depend on the type of lipid, and a slight variation in molecular structure can significantly alter the lipid binding to albumin and hence the surface physical chemical properties, as reflected in surface tension. Con-

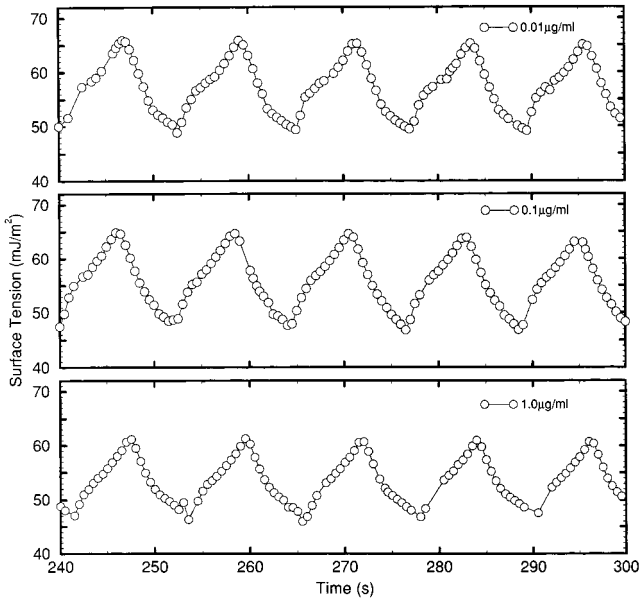

Figure 5—Dynamic surface tension response to the same area variation as that in Figure 2 for the BSA solution to which 12(*S*)-HETE-methyl ester had been added at three concentrations of 0.01, 0.1, and 1.0 μg/mL. At all three concentrations a skewed pattern is seen, similar to that of the pure BSA solution.

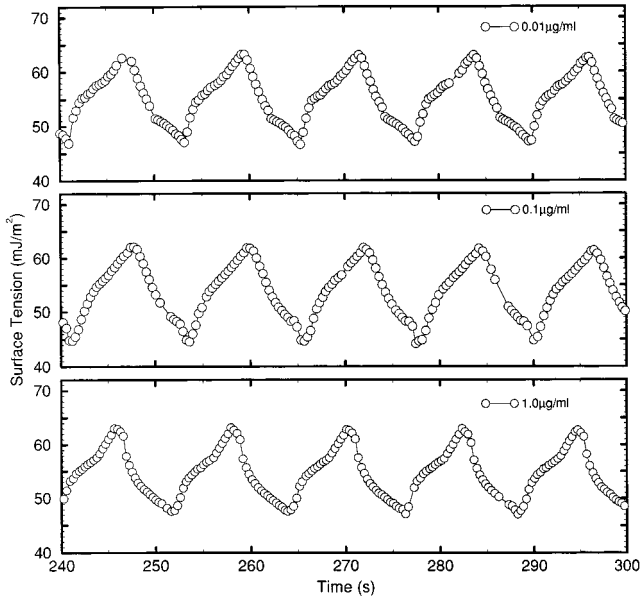

Figure 6—Dynamic surface tension response to the same area variation as that in Figure 2 for the BSA solution to which arachidonic acid-free acid had been added. Three lipid concentrations are shown: 0.01, 0.1, and 1.0 μg/mL. It was found in the experiment that at concentrations of 0.001, 0.005, and 0.05 μg/mL, the tension response is similar to that of 0.01 μg/mL. For all these concentrations, a skewed shape is always observed in the cycling tension response.

versely, surface tension measurement would provide a useful tool to differentiate lipid interactions with albumin, particularly to detect possible conformational changes as a result of the lipid binding to albumin.

Figure 6 shows the dynamic surface tension response to the same saw-tooth area variation that was used above, with arachidonic acid-free acid added to the BSA solution. A series of arachidonic acid concentrations were used: 0.001, 0.005, 0.01, 0.05, 0.1, and 1.0 μg/mL; however, only three concentrations are shown since for concentrations below 0.1 μg/mL the tension response is very similar to that of 0.01 μg/mL. Throughout these concentrations, a skewed

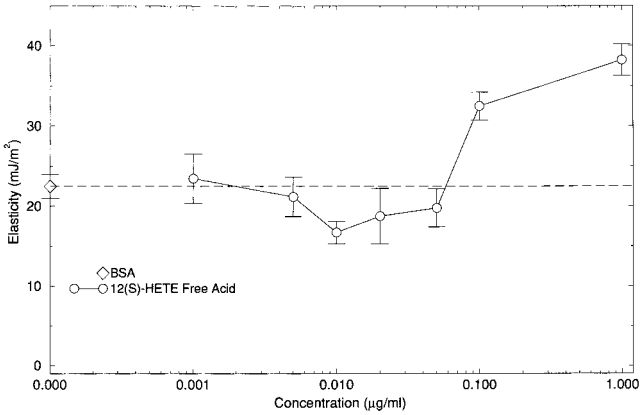

Figure 7—Surface elasticity as a function of the 12(*S*)-HETE-free acid concentration. The error bars in elasticity result from a statistical analysis, based on the Student's *t*-distribution, of a series of linear curve fits to the expansion branches of different cycles, at the 95% confidence level. There is a distinct increase in elasticity at the 12(*S*)-HETE-free acid concentration of 0.1 μg/mL, corresponding to the dose effect as observed in the dynamic surface tension response to the saw-tooth area change (Figure 3).

shape is always observed in the cycling tension response. In contrast to the observation in Figure 3 for 12(*S*)-HETE-free acid, there is no distinct change in the tension response as the arachidonic acid concentration passes 0.1 μg/mL, corresponding to a molecular ratio between lipid and protein of approximately 1:1. As previously shown with 12-(*S*)-HETE-methyl ester, Figure 6 indicates that molecular interactions between these concentrations of arachidonic acid and BSA is significantly different from those between 12(*S*)-HETE-free acid and BSA. This implies that the detected molecular binding of 12(*S*)-HETE-free acid to BSA is rather specific, and that a subtle variation in the lipid molecular structure leads to significantly different interaction properties between the lipid and the protein.

It should be noted that, in the above comparison or analysis, several less significant features are neglected, and the emphasis is placed on the high affinity binding between BSA and 12(*S*)-HETE-free acid. For example, the magnitude of changes seems slightly greater for 12(*S*)-HETE-methyl ester than for 12(*S*)-HETE-free acid at the concentration of 0.01 μg/mL (Figure 5, top panel vs Figure 3, top panel). This indicates that the interactions between the methyl ester and BSA might be slightly different from those between the free acid and BSA. Further, for the free acid, the disappearance of kinkiness at 0.1 μg/mL and higher does not seem to be complete, and a slight trace of kink remains at these concentrations. This might indicate that the high affinity interaction between the lipid and the protein does not completely block the BSA properties, although a significant change has been induced and resulted in the tension response variations. To explain all these detailed features in the tension response, more work will be conducted with respect to the mechanisms of surface molecular interactions and dynamic surface tension response to surface area changes.

C. Elasticity—As stated in the Introduction, surface elasticity is an important surface physicochemical property; it can be used as a measure of interfacial molecular interactions. The definition of the Gibbs surface elasticity, E, is

$$E = A\frac{\mathrm{d}\gamma}{\mathrm{d}A}$$

where γ is the surface tension, and A is the surface area. Figure 7 shows the surface elasticity as a function of the 12(*S*)-HETE-free acid concentration. The slope in the

surface elasticity definition was obtained through a linear curve fit to the relatively straight portion of the expansion branch, after the kink appears (Figure 3, from point A to B). The error bars in the elasticity result from a statistical analysis, based on the Student's *t*-distribution, of a series of expansion branches of different cycles, at the 95% confidence level. It can be seen that there is a distinct increase in elasticity at the 12(*S*)-HETE-free acid concentration of 0.1 μg/mL, corresponding well to the dose−effect observed in the dynamic surface tension (Figure 3). At lipid concentrations above 0.1 μg/mL, the formation of lipid−protein complexes not only changes the dynamic surface tension response to the area variation but also increases the elasticity of the adsorbed layer.

4. Conclusions

1. From the dynamic surface tension response to a patterned variation in surface area, molecular interactions between 12(*S*)-HETE-free acid and BSA have been observed.

2. The molecular interactions between 12(*S*)-HETE-free acid and BSA are concentration dependent. At the concentration corresponding to a molecular ratio of lipid to protein of 1:1, a distinct surface tension response was detected, indicating that such molecular interactions are based on one binding site for 12(*S*)-HETE on BSA. The binding appears hydrophobic in nature, i.e., the hydrophobic end of the lipid attached to a similar part of the protein.

3. At the concentration corresponding to a molecular ratio of lipid to protein of 10:1, secondary binding of 12(*S*)-HETE-free acid to BSA was detected from the dynamic surface tension response. This secondary binding appears to be hydrophilic in nature.

4. Comparison with two similar lipid molecules, 12(*S*)-HETE-methyl ester and arachidonic acid-free acid, indicates that the molecular interactions between 12(*S*)-HETE-free acid and BSA are specific.

5. Surface elasticity can be independently used for detecting molecular interactions and dose effects. In the case of 12(*S*)-HETE-free acid in BSA solution, distinctly higher surface elasticities were found at lipid concentrations in excess of the molecular ratio of lipid to protein of 1:1.

References and Notes

1. Chen, P.; Policova, Z.; Susnar, S. S.; Pace-Asciak, C. R.; Demin, P. M.; Neumann, A. W. Dynamic Surface Tension Responses to Surface Area Change of Protein/Small-Medium Organic Molecule Solutions. *Colloids Surf., A* **1996**, *114*, 99−111.
2. de Gennes, P. G. Interactions Between Polymers and Surfactants. *J. Phys. Chem*. **1990**, *94*, 8407−8413.
3. Cabane, B.; Duplessix, R. Organization of Surfactant Micelles Adsorbed on a Polymer Molecule in Water: A Neutron Scattering Study. *J. Phys*. **1982**, *43*, 1529−1542. Decoration of Semidilute Polymer Solutions with Surfactant Micelles. *J. Phys*. **1987**, *48*, 651−662.
4. Kekicheff, P.; Cabane, B.; Rawiso, M. Macromolecules Dissolved in a Lamellar Lyotropic Mesophase. *J. Colloid Interface Sci*. **1984**, *102*, 51−70.
5. Rosen, M. J. *Surfactants and Interfacial Phenomena*, 2nd. ed.; Wiley: New York, 1989.
6. Robb, I. D. Polymer/Surfactant Interactions. In *Anionic Surfactants;* Surfactant Science Series, Vol. 11; Lucassen-Reynders, E. H., Ed.; Dekker: New York, 1981; pp 109−142.
7. Gaines, G. L., Jr. *Insoluble Monolayers at Liquid−Gas Interfaces*; Interscience: New York, 1966.
8. Adamson, A. W. *Physical Chemistry of Surfaces*, 5th ed.; Wiley: New York, 1990.
9. Eijt, S. W. H.; Wittebrood, M. M.; Devillers, M. A. C. Rasing, Th. Dynamics of Protein-Surfactant Exchange at the Air−Water Interface Studied by Optical Second Harmonic Generation and Ellipsometry. *Langmuir* **1994**, *10*, 4498−4502.
10. Wahlgren, M.; Welin-Klintstroem, S.; Arnebrant, T.; Askendal, A.; Elwing, H. Competition Between Fibrinogen and a Non-Ionic Surfactant in Adsorption to a Wettability Gradient Surface. *Colloids Surf., B* **1995**, *4*, 23−31.
11. Hlady, V.; Andrade, J. D. Fluorescence Emission from Adsorbed Bovine Serum Albumin and Albumin-bound 1-Anilinonaphthalene-8-sulfonate Studied by TIRF. *Colloids Surf*. **1988**, *32*, 359−369.
12. Norde, W. Adsorption of Proteins from Solution at the Solid−Liquid Interface. *Adv. Colloid Interface Sci*. **1986**, *25*, 267−340.
13. Rotenberg, Y.; Boruvka, L.; Neumann, A. W. Determination of Surface Tension and Contact Angle from the Shapes of Axisymmetric Fluid Interfaces. *J. Colloid Interface Sci*. **1983**, *93*, 169−183.
14. Cheng, P.; Li, D.; Boruvka, L.; Rotenberg, Y.; Neumann, A. W. Automation of Axisymmetric Drop Shape Analysis for Measurements of Interfacial Tensions and Contact Angles. *Colloids Surf*. **1990**, *43*, 151−167.
15. Cheng, P. ; Neumann, A. W. Computational Evaluation of Axisymmetric Drop Shape Analysis-Profile (ADSA-P). *Colloids Surf*. **1992**, *62*, 297−306.
16. Lahooti, S.; del Rio, O. I.; Cheng, P.; Neumann, A. W. Axisymmetric Drop Shape Analysis (ADSA). In *Applied Surface Thermodynamics;* Neumann, A. W., Spelt, J. K., Eds.; Marcel Dekker Inc.: New York, N. Y., 1996; Chapter 10, pp 441−507.
17. Cabrerizo-Vílchez, M. A.; Policova, Z.; Kwok, D. Y.; Chen, P.; Neumann, A. W. The Temperature Dependence of the Interfacial Tension of Aqueous Human Albumin Solution/Decane. *Colloids Surf., B* **1995**, *5*, 1−9.
18. Chen, P.; Lahooti, S.; Policova, Z.; Cabrerizo-Vílchez, M. A.; Neumann, A. W. Concentration Dependence of the Film Pressure of Human Serum Albumin at a Water/Decane Interface. *Colloids Surf., B* **1996**, *6*, 279−289.
19. Miller, R.; Treppo, S.; Voigt, A.; Zingg, W.; Neumann, A. W. Contact Angle Kinetics of Human Albumin Solutions at Solid Surfaces. *Colloids Surf*. **1993**, *69*, 203−208.
20. Chen, P.; Kwok, D. Y.; Prokop, R. M.; del Rio, O. I.; Susnar, S. S.; Neumann, A. W. Axisymmetric Drop Shape Analysis (ADSA) and its Applications. In *Studies in Interface Science, vol. 6: Drops and Bubbles in Interfacial Research;* Möbius, D., Miller, R., Eds.; Elsevier: Amsterdam, The Netherlands 1998; pp 61−168.
21. Jyoti, A.; Prokop, R. M.; Neumann, A. W. Manifestation of the Liquid-Expanded/Liquid-Condensed Phase Transition of a Dipalmitoyl Phosphatidylcholine Monolayer at the Air−Water Interface. *Colloids Surf., B* **1997**, *8*, 115−124.
22. Prokop, R. M.; Jyoti, A.; Eslamian, M.; Garg, A.; Mihaila, M.; del Rio, O. I.; Susnar, S. S.; Policova, Z.; Neumann, A. W. A Study of Captive Bubbles with Axisymmetric Drop Shape Analysis. *Colloids Surf., A* **1998**, *131*, 231−247.
23. Zakaroff, A.; Meskini, N.; Joulain, C.; Nemoz, G.; Lagarde, M.; Prigent, A. F. 12(S)HETE Primes a Phospholipase D Pathway in Activated Human Blood Mononuclear Cells. In *Frontiers in Bioactive Lipids;* Vanderhoek, J. Y., Ed.; Plenum Press: New York, 1996; pp 291−297.
24. Meskini, N.; Zakaroff, A.; Joulain, C.; Nemoz, G.; Lagarde, M.; Pregent, A. F. Triggering of a Phospholipase D Pathway Upon Mitogenic Stimulation of Human Peripheral Blood Mononuclear Cells Enriched with 12(S)-Hydroxyicosatetraenoic Acid. *Eur. J. Biochem*. **1995**, *233*, 907−915.
25. Honn, K. V.; Tang, D. G.; Gao, X.; Butovich, I. A.; Liu, B.; Timar, J.; Hagmann, H. 12-Lipoxygenases and 12(S)-HETE: Role in Cancer Metastasis. *Cancer and Metastasis Rev*. **1994**, *13*, 365−396.
26. Pace-Asciak, C. R.; Granström, E.; Samuelsson, B. Arachidonic Acid Epoxides. *J. Biol. Chem*. **1983**, *258*, 6835−6840.
27. Peters, Th., Jr., Serum Albumin. *Adv. Protein Chem*. **1985**, *37*, 161−236.
28. Carter, D. C.; Ho, J. X. Structure of Serum Albumin. *Adv. Protein Chem*. **1994**, *45*, 153−196.
29. Huntley, T. E.; Neitzel, J. K.; Elson, M. K. Binding Properties of Purified Adult and Fetal Bovine Serum Albumin. *Biochim. Biophys. Acta* **1977**, *490*, 112−119.

Acknowledgments

This research was supported financially by the Medical Research Council of Canada to A.W.N. (MT 5462) and to CPR-A (MT 4181).

JS9901256