

Adsorptive Behavior of a Globular Protein with a Monoglyceride Monolayer Spread on the Aqueous Surface

Daechul Cho[†] and Michel Cornec

Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907-1146, U.S.A.

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Abstract-The dynamics of surface pressure (p) and of surface concentration (G) of a ^{14}C radiolabeled *b*-lactoglobulin, a globular protein, adsorbed onto a spread monolayer of 1-monopalmitoyl-rac glycerol (named commonly as a monoglyceride), at the air-water interface were measured. The adsorption of ^{14}C labeled *b* lactoglobulin was enhanced at short times when C-16 monoglyceride of 73.4 and 24.7 μg was spread on the aqueous surface. However, the amounts of protein adsorbed at the steady state (after 10 h) were lowered with the values being 0.8 and 1.2 mg/m^2 , respectively. Spreading of such amount of monoglyceride that forms a dense packed monolayer onto air-water interface led to complete displacement of the protein from the aqueous surface after 2.5 h, possibly because of the surface pressure and exclusion effects.

Key words : Lipid-Protein Interaction, Monolayer, Monoglyceride, *b*-Lactoglobulin

INTRODUCTION

Emulsions and foams are commonly found in various applications such as pharmaceutical, waste-treatment, oil mining and food processing industries. Such dispersions are thermodynamically unstable in their practical uses, but their stability may be enhanced by the adsorption of amphiphilic molecules to the surface of the dispersed phase as seen in Fig. 1 [Israelachvili, 1994]. These molecules generally fall in two classes, macromolecules such as proteins and low molecular weight surfactants such as monoglycerides and phospholipids. Surfactants adsorb and orient at fluid-fluid interfaces, and thus reduce the interfacial tension between the phases. Proteins form a condensed viscoelastic film of highly self-interacting molecules at the interface which resist local deformation [Clark et al., 1994]. In contrast, surfactants form a fluid adsorbed layer in which adsorbed molecules can diffuse laterally toward regions of high surface tension conferring stability via the Marangoni effect [Ewers and Sutherland, 1952]. Individually, the viscoelastic and Marangoni mechanisms are very effective at stabilizing foams and emulsions but are mutually incompatible. Low molecular weight surfactants, because of their much lower molecular weight, pack more efficiently at the interface and thus reduce the interfacial tension to a greater extent than proteins [McClements, 1998]. On the other hand, intermolecular interactions between low molecular weight surfactant are much weaker and development of high mechanical strength is not possible. For these reasons, both classes of molecules are used in many biological systems. This may cause a problem as competition between the above two mechanisms arises, leading to

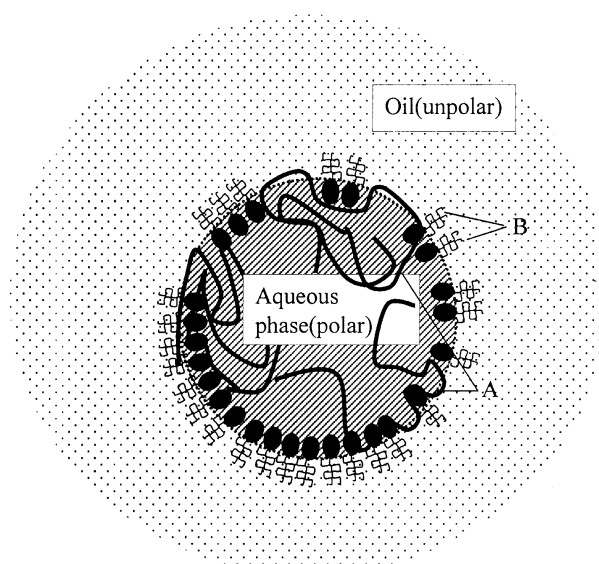


Fig. 1. An example of emulsion (water in oil) stabilized with stabilizers such as macro amphiphilic molecules (A) and small molecules with polar heads and hydrocarbon chains (B).

instability of the system [Clark et al., 1991; Coke et al., 1990; Cornec et al., 1996; Courthaudon et al., 1991; Sarker et al., 1995; Wilde and Clark, 1993].

Despite a considerable amount of work, little is known, in a quantitative sense, about the molecular mechanisms of protein-surfactant interactions at the surface. Surface properties (structure, stability, mechanical and dynamic properties) of protein-lipid complexes depend on the mechanisms of protein-lipid interactions at the surface. Reported mechanisms for protein-surfactant interactions involved either electrostatic interactions between ionic surfactant head group and the charged macromolecules [Nylander, 1998; Cornell and Patterson, 1989; Kozorac

[†]To whom correspondence should be addressed.

E-mail : dcho@lgen.co.kr

Current address : LG Corporate Inst. of ESH, Yonsei Engineering Research Center, Yonsei University, Seoul 120-749, Korea

et al., 1988; Quinn and Dawson, 1969; Cornell et al., 1990; Brewkink et al., 1992; Grimard et al., 1993] and/or hydrophobic interactions [Bos and Nylander, 1996; Du et al., 1996]. Electrostatic interactions were studied by Cornell et al. [Cornell and Patterson, 1989; Cornell and Carroll, 1985]. Using the film balance technique, they investigated the adsorption of various whey proteins from solution into a spread monolayer of phospholipids. They observed that the amount of protein bound to the lipid was more important when both molecules carry opposite net charges and this amount was decreased with an increase of the ionic strength of the solution. They also found that penetration of protein into mixed monolayer of phospholipids can occur at higher surface pressure than the equilibrium surface pressure obtained for the adsorption of the proteins and they concluded that the formation of pure protein patches was unlikely and that portions of the protein were intercalated in the lipid monolayer. On the other hand, Du et al. [1996] showed that the penetration of an enzyme, the glucooxydase, into a glycolipid monolayer was easier for longer aliphatic chain of the glycolipid. Their results suggest that the hydrophobic interaction is the predominant force in the interactions. Formation of protein-lipid complexes may result in a structural change of the former component [Cornell, 1982; MacRitchie, 1978] which can affect the structure of the adsorbed layer as well as the film formation capacity and thus, such formation led to the formation and stability of emulsions and foams. In addition, the physical state of the lipid phase has been shown to be important for film formation. It was observed that protein adsorbs to a greater extent to expanded lipid monolayers than to condensed ones [Ibdah and Phillips, 1988]. In addition, it was observed that lipids which form condensed films at the air-water interface do not form homogeneous films with proteins. On the other hand, those exhibiting liquid-expanded monolayer behavior tend to produce well-mixed monolayers with proteins [Rodriguez et al., 1998].

Most of the previous work on mixed lipid-protein monolayer has focussed on the interactions between protein and polar phospholipids. Less attention has been directed towards mixed monolayers formed with protein and monoglycerides [Rahman and Sherman, 1982; Rodriguez and Rodriguez, 1998] despite the fact that they are typical emulsifiers or stabilizers in most biological systems.

In this study, a mixed system of *b*-lactoglobulin and 1-monopalmitoyl-rac glycerol was studied. Changes in surface concentration of the proteins were probed by using ^{14}C radiolabelled proteins.

EXPERIMENTAL

1. Materials and Apparatus

b-Lactoglobulin and 1-monopalmitoyl-rac glycerol (C16:0) were purchased from Sigma Chemical Inc. (St. Louis, MO). Reagent grade *n*-hexane and ethanol were purchased from Aldrich Chemical. Monoglyceride solutions were made in hexane-ethanol (v/v=9:1) and were used within two days. Isotopes of [^{14}C]formaldehyde (37.3 %) and [^{14}C]sodium stearate were purchased from Sigma Chemical Inc. Sodium cyanoborohydride

(NaCNBH_3) was purchased from Aldrich Chemical Inc. All the experiments were carried out at pH 7.4 using a 10 mM commercial sodium phosphate buffer containing 0.9 % NaCl. Throughout the experiments, ultrapure deionized water was used.

A Langmuir minitrough (with dimensions of 330×75×6.5 mm) from KSV (Helsinki, Finland) was used for both surface pressure and surface concentration measurements. A gas proportional detector (Ludlum model 120, with a 2×2 in Mylar window) with a digital scaler/counter (Ludlum model 520) was used for detecting radioactivity, in counts per minute (CPM), from the adsorbed monolayer at the air-water interface. Radioactivity was measured under P-10 gas (10 % methane in argon) flowing at 55 ml/min through the detector chamber.

2. Methods

2-1. Radiolabelling of Proteins

b-Lactoglobulin (30 mg) was dissolved in 0.05 M phosphate buffer (pH 7) and mixed with 0.1 M sodium cyanoborohydride and [^{14}C]formaldehyde (102 mCi) and allowed to react for 2 hours at room temperature [Hunter et al., 1991]. After the reaction, the mixture was dialyzed against a 0.05 M phosphate buffer for 30 hours at 4 °C for complete removal of unreacted species. *b*-Lactoglobulin was found to have one amide group labeled per molecule on the average (2.52 mCi/mg of protein, respectively) as analyzed with a scintillation counter (Model Tri-carb 4000, from Packard Instruments Inc.). Since the degree of modification due to radiolabelling is small, it was assumed that the surface properties of the protein were not affected significantly. Comparison of the spread monolayer isotherm of native and radiolabelled bovine serum albumin as reported by Cho et al. [1997] indicated no significant differences in the surface activity between the two because of radiolabelling.

2-2. Surface Pressure-Molecular Area (*p*-A) Isotherms

The Langmuir trough was first filled with phosphate buffer. Then, the surface was cleaned by sweeping it with the Teflon barrier, and any surface-active contaminants were removed by suction (aspiration) of the interface. The lipid (lecithin or monoglyceride) was spread over the clean-air water interface by applying the solution dropwise from a Hamilton syringe. Protein spread monolayers were prepared using the Trumit's monolayer spreading method. Aliquots of 50 ml of a 0.0247 wt% protein solution were dripped from the top of a glass rod (5 mm diameter and 5 cm long) positioned across the air-water interface. The solution was spread uniformly on the interface.

For mixed monolayers, first *b*-lactoglobulin monolayer was formed with Trumit's method and allowed to rest for 10 minutes. Then, various aliquots of lipid solution in hexane-ethanol were spread at several spots on the surface. The monolayers were allowed to equilibrate for another 10 minutes. Then, the surface areas was compressed by moving Teflon barriers at a constant speed of 4 cm²/min and the surface pressure was continuously recorded. Immediately after the end of the compression stage, the area was expanded at the same rate.

2-3. Adsorption from Solution

The trough was filled with the buffer solution without any protein (surface tension=*g*) and the surface was carefully aspirated to remove any surface impurities before the surface

pressure was adjusted to zero ($p = g - g$). Then, the protein solution was gently poured into the trough. After that a lipid monolayer was spread using a Hamilton syringe and the surface pressure and surface concentration (via radioactivity measurements) were monitored up to 10 hours. In another set of experiments, the lipid was spread on top of adsorbed protein monolayers prepared by allowing the protein to adsorb for 150 min.

2-4. Calibration of Ludlum Gas Proportional Detector

The CPMs were converted to surface concentrations by calibrating the Ludlum gas proportional detector with radioactive samples of known surface or bulk concentrations. The bulk radioactivity calibration procedure was referred to that of Hunter's [Hunter et al., 1991]. [^{14}C] *b*-lactoglobulin itself, rather than [^{14}C] stearic acid, was used for calibration of the surface radioactivity, since the use of [^{14}C] stearic acid tends to underestimate the surface concentration due to its much smaller molecular size compared to the proteins [Cho et al., 1997; Xu and Damodaran, 1993]. A total of 175 ml of 0.01 M phosphate buffer (pH 7, containing 0.9 % NaCl) was poured into the Langmuir mini-trough and a spread monolayer of [^{14}C] protein was formed using Trurnit's method. The detector was placed at a distance of 3 mm above the air-water interface and the steady state CPM was measured. The area of the air-water interface was compressed in stages to provide several surface radioactivities, which were used, for the detector calibration.

RESULTS

1. Surface Isotherms

π - \bar{A} isotherms of *b*-lactoglobulin and 1 monopalmitoyl-rac glycerol are shown on Fig. 2 (a-b). *b*-Lactoglobulin monolayer behaved like gas for \bar{A} greater than 4,000 $\text{\AA}^2/\text{molecule}$, like liquid for \bar{A} between 1,400 and 2,700 $\text{\AA}^2/\text{molecule}$. At about 22 mN/m, an inflection point is observed in the isotherm. Above this π , whole protein molecules or segments of the protein are probably squeezed down into the subphase. The fact that the compressed monolayer was able to recover to its initial state upon decompression and that no shift in the molecular area occurred upon subsequent compression ruled out massive loss of polypeptide molecule to the subphase. However, the observed hysteresis suggests that the recovery is slow. Similarly, as observed for BSA, the value of \bar{A} at maximum compression (763 $\text{\AA}^2/\text{molecule}$ at 27 mN/m) was close to the theoretical minimum area of *b*-lactoglobulin ($\bar{A}=1,000 \text{\AA}^2/\text{molecule}$ for a sphere of dimensions 3.58×3.58 nm).

The isotherm of the C-16 monoglyceride monolayer showed a gradual increase in the surface pressure until a plateau occurred at between 40 and 60 $\text{\AA}^2/\text{molecule}$. The plateau reveals a phase transition from a liquid expanded (LE) state to a liquid condensed (LC) state. Gehlert et al. [1995], using Brewster Angle Microscopy (BAM) observed in the region corresponding to the beginning of the plateau some condensed phase domains surrounded by a homogeneous fluid phase of low density. As the molecular area was decreased, the domains grew in area at the expense of the fluid phase. Upon further compression, the domains started to overlap, filling the gaps in the condensed

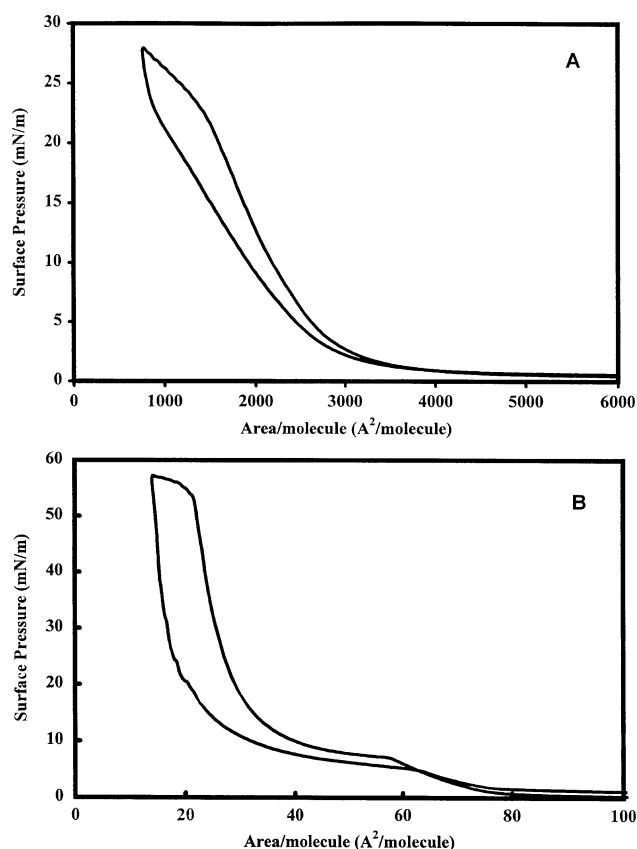


Fig. 2. π - \bar{A} (Area per molecule) isotherm of (a) *b*-lactoglobulin (b) 1 monopalmitoyl-rac glycerol (C16:0).

phase, which was accompanied by an increase in the surface pressure. Above a surface pressure of 25 mN/m, the domains were compressed to close packing without visible gap. Hysteresis was also observed for C16 monoglyceride monolayers with \bar{A} being smaller at a certain π during the expansion than the compression cycle.

Mixed monolayer isotherms are shown in Fig. 3 for weight ratios of 1 monopalmitoyl-rac glycerol to *b*-lactoglobulin ($R_{\text{C16/b-lg}}$) from 3 to 0.33. Analysis of the shape of the isotherms obtained shows the effect of the composition on the surface pressure and on the area during compression. An LE-LC transition is visible on all isotherms at about 7-8 mN/m. The shift of the transition to higher areas when the proportion of *b*-lactoglobulin is increased points out the presence of the two constituents at the interface. Nevertheless, the conservation of the LE-LC transition is evidence of the great influence of the monoglyceride on the properties of the mixed monolayer during compression [Boury et al., 1995a; Boury et al., 1995b]. At higher surface pressures, the mixed isotherms exhibited another transition, which was also present in the isotherm of the pure *b*-lactoglobulin. C16-monoglyceride-rich monolayers exhibited smoother phase transitions than *b*-lactoglobulin-rich monolayers. At the lowest $R_{\text{C16/b-lg}}$ ratio, the transition was reduced to a kink, which may correspond to the expulsion of the protein molecules from the mixed film resulting in the reduction of the compressibility of the film to a level similar to that of pure monoglyceride. As the film was further compressed, the

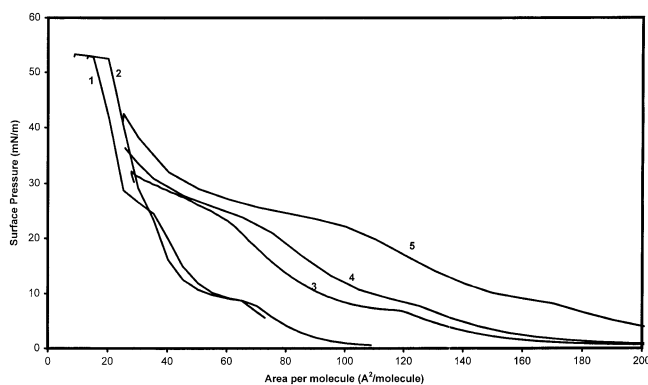


Fig. 3. p - \bar{A} isotherms of mixed monolayers for varied M/B mass ratio.

curve 1: M/B=3, curve 2: M/B=2, curve 3: M/B=1, curve 4: M/B=0.5, curve 5: M/B=0.33. The average molecular area of the mixture, $\bar{A} = A/(n_L + n_{b-lg})$ where A is the total area, n_L and n_{b-lg} are the total number of molecules of lecithin and *b*-lactoglobulin, respectively.

isotherms of the mixed monolayer asymptotically approached that of the monoglyceride monolayer. At high enough surface pressure, the isotherms for different compositions of the mixed monolayer coincided, which indicated that *b*-lactoglobulin molecule was completely squeezed out from the 1 monopalmitoyl-rac glycerol monolayer eventually. However, the collapse surface pressure was observed to be lower for mixed monolayers than for pure monoglycerides. Hysteresis was observed for C16 monoglyceride and *b*-lactoglobulin mixed monolayers (results not shown) with the average molar areas being smaller during compression cycle.

2. Absorption and Exchange for Protein-Lipid Surfactant Mixtures

Mutual interaction of protein-lipid was investigated by spreading a lipid monolayer on an adsorbing protein solution. First, control experiments were performed in order to validate the methodology used in this study. Spreading of solvent only (hexane-ethanol mixture without any lipid) on an adsorbing protein solution resulted in a small overshoot in p but no significant change in G was noted. It was concluded that the spreading solvent did not significantly affect protein adsorption. The second control experiment consisted at spreading 50 μg of lipid in solvent on a clean interface. A sudden increase in p with a small overshoot was observed. Since p was observed to remain constant after spreading, it was concluded that the spread lipid did not experience any desorption. Therefore, no attempt was made to separately monitor the lipid surface concentration, which was assumed to be constant.

Spreading 12.35 μg (110 $\text{\AA}^2/\text{molecule}$) of 1 monopalmitoyl-rac glycerol on a nearly clean interface was observed to slightly enhance the adsorption of *b*-lactoglobulin ($G=1.82 \text{ mg/m}^2$ instead of 1.63 mg/m^2 when no lipid monolayer was at the interface). As observed previously, the initial rate of adsorption was increased. Increasing the amount of C16 monoglyceride spread at the surface caused the steady state surface concentration of *b*-lactoglobulin to be decreased. Indeed, G was 1.19 and 0.86 mg/m^2 , respectively when 24.7 μg (27.5 $\text{\AA}^2/\text{mole-}$

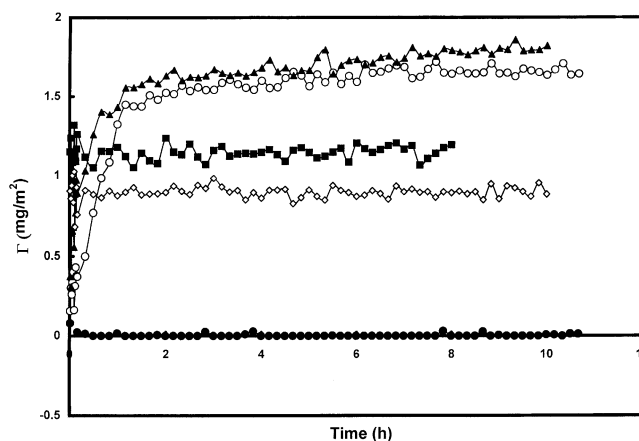


Fig. 4. Time dependent changes of G when a solution of $[^{14}\text{C}]\textit{b}$ -lactoglobulin ($c_b=1 \text{ ppm}$) was poured in the trough, followed by immediate spreading of 1 monopalmitoyl-rac glycerol (C16:0).

\circ : no monoglyceride spread, \triangle : 12.35 μg (110 $\text{\AA}^2/\text{molecule}$), \square : 24.7 μg (27.5 $\text{\AA}^2/\text{molecule}$), \triangle : 73.4 μg (19 $\text{\AA}^2/\text{molecule}$), \bullet : 113 μg (12 $\text{\AA}^2/\text{molecule}$).

cule) and 73.4 μg (19 $\text{\AA}^2/\text{molecule}$) were spread (Fig. 4). However, it is to be noted that the initial rate of adsorption was faster as the spread amount of monoglyceride was increased. Protein was excluded from the interface when a close pack monolayer (12 $\text{\AA}^2/\text{molecule}$) of C16 monoglyceride was present at the interface.

The response to an adsorbed *b*-lactoglobulin monolayer to the spreading of a monoglyceride monolayer was also tested by spreading different amounts of monoglycerides on an adsorbing protein solution. Results are shown on Fig. 5. As observed previously for BSA/lecithin mixtures [Cho et al., 1997], the effect of the spread monoglyceride monolayer on the adsorption of *b*-lactoglobulin depended on the amount of monoglyceride spread. Protein adsorption was found to be enhanced

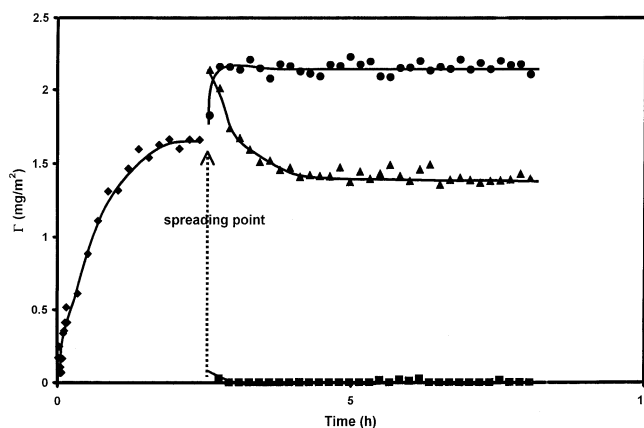


Fig. 5. Time-dependent changes of G for 1 ppm $[^{14}\text{C}]\textit{b}$ -lactoglobulin ($c_b=1 \text{ ppm}$) solution (\blacklozenge).

At $t=150 \text{ min}$, \square : 24.7 μg (27.5 $\text{\AA}^2/\text{molecule}$), \triangle : 73.4 μg (19 $\text{\AA}^2/\text{molecule}$), \blacksquare : 113 μg (12 $\text{\AA}^2/\text{molecule}$) of 1 monopalmitoyl-rac glycerol (C16:0) was spread (upward arrow points out) at the surface.

when 24.7 μg (27.5 $\text{\AA}^2/\text{molecule}$) was spread at the surface. If this amount was increased to 73.4 μg (19 $\text{\AA}^2/\text{molecule}$), the amount of protein adsorbed was first increased followed by a sudden desorption and G reached a steady state value of 1.40 mg/m^2 . **b**-Lactoglobulin was found to be displaced from the interface by spreading 113 μg (12 $\text{\AA}^2/\text{molecule}$) of monoglyceride.

DISCUSSION

Spreading a lipid monolayer of monoglyceride onto an adsorbing protein solution of **b**-lactoglobulin resulted in an enhancement in the adsorption of the protein soon after the lipid surfactant was spread. This was evidenced by the increase in the initial rate of the protein adsorption. However, when the amount of lipid spread at the interface was increased, the steady state surface concentration of the protein was observed to be lower than the one observed for protein when no surfactant was at the interface.

The initial enhancement in the protein adsorption, soon after spreading of the lipid, may be due to the formation of a complex between the hydrophobic patches of the protein and the hydrophobic chains of the lipid molecules [Du et al., 1996]. Such a behavior can be explained in terms of the adsorption energy E_{ad} of a protein molecule [Cho et al., 1997] which can be written as :

$$E_{ad} = E_{sp} + E_{el} + E_{hy} \quad (1)$$

where E_{sp} is the work that needs to be done by an adsorbing protein molecule to anchor itself at the interface and acts against the surface pressure energy barrier due to the steric interactions of the molecules already present at the interface. E_{el} is the electrostatic energy due to the formation of a electric double layer subsequent to the adsorption of protein molecules at the interface. E_{hy} is the hydrophobic energy due to the exposure of the hydrophobic patches of the protein into air [Narsimhan and Uraizee, 1992].

E_{sp} and E_{el} are expected to be positive and will oppose the protein adsorption, whereas E_{hy} is negative and will promote adsorption. Protein may form a complex with lipid molecule if E_{hy} is greater than the sum $E_{sp} + E_{el}$. With the presence of a close-packed lipid monolayer at the interface, protein may be expelled from the interface because the contribution of E_{sp} is dominant.

Based on the average surface hydrophobicity and molecular dimensions of the protein molecules, the estimated value of E_{hy} is of the order of 250 kJ/mol for **b**-lactoglobulin. And the estimated values of E_{el} were found to be negligible at any protein surface concentration (5.8 kJ/mol at the highest value of $G \approx 2.18 \text{ mg}/\text{m}^2$). E_{sp} was estimated to be on the order of 105 and 275 kJ/mol when p was 18 and 47.5 mN/m, respectively (corresponding to a spread monolayer of monoglyceride of 27 and 12 $\text{\AA}^2/\text{molecule}$, respectively). Consequently, E_{sp} is much higher than E_{hy} when a close-packed monolayer of monoglyceride is spread at the surface and therefore **b**-lactoglobulin molecules are expelled from the interface.

CONCLUSIONS

This paper has determined p - A isotherms of spread monolayers of 1 monopalmitoyl-rac glycerol, **b**-lactoglobulin and mixtures of monoglyceride/ β -lactoglobulin at ambient temperature. Also, the dynamics adsorption of [^{14}C] **b**-lactoglobulin for different spread amounts of monoglyceride and time delays after the initiation of protein adsorption was investigated. The conclusions are as follows :

1. All mixed 1 monopalmitoyl-rac glycerol/**b**-lactoglobulin isotherms exhibited a phase transition between a liquid expanded state to a liquid condensed state, which indicates that the monoglyceride has a strong influence on the properties of the mixed monolayer during compression.
2. The isotherm of the mixed monolayer approached asymptotically that of the lipid monolayer thus indicating that the protein is squeezed out from the monolayer at very small areas of the monolayer.
3. Lipid-rich monolayers exhibited hysteresis and smoother phase transition than protein-rich monolayers.
4. Spreading a low amount of lipid at the interface resulted in an enhancement of the protein adsorption at short times leading to a protein surface concentration higher than the steady state value in absence of lipid.
5. Spreading higher amount of lipids decreased the amount of protein adsorbed and spreading a close-packed lipid monolayer eventually caused desorption of protein from the interface, maybe because of the surface pressure and steric exclusion effects produced by the spread lipid monolayer.

REFERENCES

- Bos, M. A. and Nylander, T., "Interaction between **b**-Lactoglobulin and Phospholipids at the Air-Water Interface," *Langmuir*, **12**, 2791 (1996).
- Boury, F., Ivanova, I., Panaiotov, I. and Proust, J. E., "Dilatational Properties of poly(D, L-lactic acid) and Bovine Serum Albumin Monolayers Spread at the Air/Water Interface," *Langmuir*, **11**, 599 (1995).
- Boury, F., Ivanova, I., Panaiotov, I. and Proust, J. E., "Dilatational Properties of poly(D, L-lactic acid) and Bovine Serum Albumin Monolayers Formed from Spreading an Oil-in-Water Emulsion at the Air-Water Interface," *Langmuir*, **11**, 2131 (1995).
- Brewkink, E., Demel, R. A., De Korte-Kool, G. and De Kruijff, B., "SecA Insertion into Phospholipids is Stimulated by Negatively Charged Lipids by ATP: A Monolayer Study," *Biochemistry*, **31**, 1119 (1992).
- Cho, D., Narsimhan, G. and Franses, E. I., "Interactions of Spread Lecithin Monolayers with Bovine Serum Albumin in Aqueous Solution," *Langmuir*, **13**, 4710 (1997).
- Clark, D. C., Mackie, A. R., Wilde, P. J. and Wilson, D. R., "Differences in the Structure and Dynamics of the Adsorbed Layers in Protein-Stabilized Model Foams and Emulsions," *Faraday Discuss*, **98**, 253 (1994).
- Clark, D. C., Wilde, P. J. and Wilson, D. R., "Competitive Adsorp-

- tion in Oil-in-Water Emulsions Containing α -Lactalbumin and β -Lactoglobulin," *Colloids and Surfaces*, **59**, 209 (1991).
- Coke, M., Wilde, P. J., Russel, E. J. and Clark, D. C., "The Influence of Surface Composition and Molecular Diffusion on the Stability of Foams Formed from Protein/Surfactant Mixtures," *J. Colloid Interface Sci.*, **138**, 489 (1990).
- Corneć, M., Mackie, A. R., Wilde, P. J. and Clark, D. C., "Competitive Adsorption of β -Lactoglobulin and β -Casein with Span 80 at the Oil-Water Interface and the Effects on Emulsion Behaviour," *Colloids Surfaces A: Physicochem. Eng. Aspects*, **114**, 237 (1996).
- Cornell, D. G. and Carroll, R. J., "Miscibility in Lipid-Protein Monolayers," *J. Colloid Interface Sci.*, **108**, 226 (1985).
- Cornell, D. G. and Patterson, D. L., "Interaction of Phospholipids in Monolayers with β -Lactoglobulin Adsorbed from Solution," *J. Agric. Food Chem.*, **37**, 1455 (1989).
- Cornell, D. G., "Lipid-Protein Interactions in Monolayers: Egg Yolk Phosphatidic Acid and β -Lactoglobulin," *J. Colloid Interface Sci.*, **88**, 536 (1982).
- Cornell, D. G., Patterson, D. L. and Hoban N., "The Interaction of Phospholipids in Monolayers with Bovine Serum Albumin and α -Lactalbumin Adsorbed from Solution," *J. Colloid Interface Sci.*, **140**, 428 (1990).
- Courthaudon, J. L., Dickinson, E., Matsumara, Y. and Clark, D. C., "Competitive Adsorption of β -Lactoglobulin+Tween 20 at the Oil-Water Interface," *Colloids Surfaces*, **56**, 293 (1991).
- Du, Y., An, J., Tang, J. and Jiang, L., "A Study of the Interaction between Glycolipids of Different Hydrophobicities and Glucose Oxidase by a Monolayer Technique," *Colloids and Surfaces B*, **7**, 129 (1996).
- Ewers, W. E. and Sutherland, K. L., "The Role of Surface Transport in the Stability and Breakdown of Foams," *Aust. J. Sci. Res. Ser. A*, **5**, 697 (1952).
- Gehlert, U., Weidemann, G. and Vollhardt, D., "Morphological Features in 1-Monoglyceride Monolayers," *J. Colloid Interface Sci.*, **174**, 392 (1995).
- Grimard, R., Tancrede, P. and Gicquaud, C., "Interaction of Actin with Positively Charged Phospholipids: a Monolayer Study," *Biochem. Biophys. Res. Com.*, **190**, 1017 (1993).
- Hunter, J. R., Kilpatrick, P. K. and Carbonell, R. G., " β -Casein Adsorption at the Air/Water Interface," *J. Colloid Interface Sci.*, **142**, 429 (1991).
- Ibdah, J. A. and Phillips, M. C., "Effect of Lipid Composition and Packing on the Adsorption of Apolipoprotein A-I to Lipid Monolayers," *Biochemistry*, **27**, 7155 (1988).
- Israelachvili, J., "The Science and Applications of Emulsions-an Overview," *Colloids and Surfaces A*, **91**, 1 (1994).
- Kozorac, Z., Dhathathregan, A. and Möbius, D., "Adsorption of Cytochrome c to Phospholipid Monolayers Studied by Reflection Spectroscopy," *FEBS*, **229**, 372 (1988).
- MacRitchie, F., "Spread Monolayers of Proteins," *Adv. Protein Chem.*, **32**, 283 (1978).
- McClements, D. J., "Food Emulsions: Principles, Practice and Techniques," CRC Press (1998).
- Narsimhan, G. and Uraizee, F., "Kinetics of Adsorption of Globular Proteins at an Air-Water Interface," *Biotechnol. Prog.*, **8**, 187 (1992).
- Nylander, T. in "Proteins at Liquid Interfaces," Möbius, D. and Miller, R. Eds., Elsevier (1998).
- Quinn, P. J. and Dawson, R. M. C., "The Interaction of Cytochrome c with Monolayers of Phosphatidylethanolamine," *Biochem J.*, **113**, 791 (1969).
- Rahman, A. and Sherman, P., "Interaction of Milk Proteins with Monoglycerides and Diglycerides," *Colloid & Polymer Sci.*, **260**, 1035 (1982).
- Rodriguez Nino, M. R. and Rodriguez Patino, J. M., "Surface Tension of Bovine Serum Albumin and Tween 20 at the Air-Water Interface," *JAOCs*, **75**, 1233 (1998).
- Rodriguez Nino, M. R., Wilde, P. J., Clark, D. C. and Rodriguez Patino, J. M., "Surface Dilational Properties of Protein and Lipid Films at the Air-Water Interface," *Langmuir*, **14**, 2160 (1998).
- Sarker, D. K., Wilde, P. J. and Clark, D. C., "Competitive Adsorption of L- α -Lysophosphatidylcholine/ β -Lactoglobulin Mixtures at Interfaces of Foams and Foam Lamellae," *Colloids Surfaces B: Biointerfaces*, **3**, 349 (1995).
- Wilde, P. J. and Clark, D. C., "The Competitive Displacement of β -Lactoglobulin by Tween 20 from Oil-Water and Air-Water Interfaces," *J. Colloid Interface Sci.*, **155**, 48 (1993).
- Xu, S. and Damodaran, S., "Calibration of Radiotracer Method to Study Protein Adsorption at Interfaces," *J. Colloid Interface Sci.*, **157**, 485 (1993).