Surface Properties of β-Lactoglobulin: Adsorption and Rearrangement during Film Formation

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The surface activity of β-lactoglobulin, β-LG (0.10 g/mL), as a function of time and pH was studied using a Wilhelmy plate method. Surface pressure increased rapidly during the initial 60 min and slowly reached an equilibrium value of 25–27 mN/M after 360 min at pH >5.0. The rate of adsorption and packing of β-LG in the interfacial film was maximum near the isoelectric point. Appropriate calculations revealed that the relaxation times of adsorption and rearrangement were <5 min and >3 h, respectively, and were sensitive to pH. Data corresponding to the area cleared per protein molecule in the interface and the work of compression were estimated. The values increased with time and varied with pH in range 3.3–7.3, indicating the importance of electrostatic effects in formation and properties of the film.

INTRODUCTION

In many foods, proteins facilitate the manufacture and improve several quality attributes by virtue of their unique physicochemical properties. These properties are influenced by chemical and structural characteristics of the protein, i.e., the content and disposition of amino acid residues, molecular size, shape and flexibility, secondary conformations, net charge, etc., as well as by the particular conditions prevailing in the system and interactions of proteins with other components (Kinsella, 1976, 1982a,b). Many formulated foods exist structurally as foams or emulsions wherein proteins aid their formation and stabilization by virtue of their surface activity (MacRitchie, 1978; Halling, 1981; Kinsella, 1981; Waniska and Kinsella, 1981). Surface-active agents lower the surface tension of liquids by adsorbing at the air–liquid interface even at relatively low concentrations; e.g., the surface tension of water is significantly decreased by the presence of 10^{-5}% casein (Phillips et al., 1975, 1979a). The surface activity or interfacial properties of proteins have been reviewed by Kitchener and Cooper (1959), Davies and Rideal (1963), MacRitchie and Alexander (1963a,b), Alexander and Hibberd (1971), Joly (1972a,b), and Goodrich (1973). These authors indicated that the surface properties of protein solutions affect their foaming and emulsification properties. Several authors (MacRitchie, 1978; Graham and Phillips, 1979 a,b,c; Waniska and Kinsella, 1979, 1981; Halling, 1981; Kinsella, 1981, 1982a) have discussed some chemical and structural characteristics of the proteins that affect their surface properties. However, the relationships between the chemical characteristics of protein, i.e., the hydrophobic, ionic, and hydrophilic characteristics, and the foaming properties of the protein are not well understood. Current hypotheses suggest that the hydrophobic characteristics of proteins are related to surface activity, foaming, and emulsification properties (Birdi, 1973f Horiuchi et al., 1978; Kato and Nakai, 1980; Nakai et al., 1980; Graham and Phillips, 1979c; Kinsella, 1982a). Earlier hypotheses indicated that the hydrophilic interactions between the proteins and the aqueous phase were also important for surface properties (Cumper, 1953; German et al., 1985).

In order to determine the importance of surface charge and hydrophilicity on interfacial and film-forming properties, we studied the interfacial properties of β-lactoglobulin (β-LG) because its molecular properties are well characterized (McKenzie, 1971). In this paper we describe methodology and the adsorption and film-forming behavior of β-LG. The corresponding properties of chemically modified β-LG will be described subsequently.

METHODS AND MATERIALS

Bovine β-LG (crystallized and lyophilized; lot no. 118C-8015; Sigma Chemical Corp., St. Louis, MO) was dialyzed in 205-cm-diameter dialysis tubing (Fisher Scientific Co., Rochester, NY) against doubly distilled, deionized water containing 0.01% sodium azide and lyophilized before use. The water used for the surface activity measurements was twice distilled, the second time from a glass apparatus.

The surface properties of β-LG were investigated by measuring the change in surface tension of solutions over time at different pHs. The methods for measuring the surface tension of liquids have been reviewed by Padday (1969). The surface tension of protein solutions approaches equilibrium after many hours (Adamson, 1975). Hence, there are only a few practical methods available for measurement of surface tension of protein solutions, viz. the sessile drop, the drop volume, and the Wilhelmy plate methods. The sessile drop method is tedious; the drop volume method is best suited for proteins that come to equilibrium very rapidly (Tornberg, 1978a). Hence, in this study we used the Wilhelmy plate method (Davies and Rideal, 1963; Adamson, 1975), which was amenable to monitoring surface tension (γ) over long time periods though it was unsuitable for measuring the initial phases (<15 s) of protein adsorption.

The surface tension was determined by using a Cahn electrobalance, Model 2000, and a Cahn Instruments platinum sensor (1.0 × 1.8 × 0.05 cm), Model No. 269 (Ventron Corp., Ceritos, CA). The electrobalance was calibrated with the platinum sensor in place using a 100.00 ± 0.005 mg weight. Other instruments used were a micrometer (Model No. 436-25 mm; L. S. Starrett Co., Athol, MA), an automatic pipetter (Gilson Pipetman, Model P5000; Rainin Instruments Co. Inc., Brighton, MA), and a linear recorder (Pye Unicam AR25; W. G. Pye & Co. Ltd., Cambridge, England). The instrument was maintained in a plastic glovebox (75 × 45 × 35 cm) to maintain a constant temperature (23 °C) and humidity (95% RH) during surface tension measurements. The platinum plate was carefully cleaned before use with fine steel wool to roughen the surfaces. The platinum sensor was rinsed with acetone and allowed to air-dry before flaming in a bunsen burner several times to remove organic materials. After cooling, the sensor was positioned on a hooked wire attached to the microbalance in the glovebox. The dishes used in this analysis were cleaned according to the method of Turner

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et al. (1974), i.e., thoroughly washed with a solution of 0.1 N potassium hydroxide in 95% ethanol. The dishes were rinsed thoroughly with distilled water and air-dried.

The β-LG protein solution (0.12% (g/mL)) was prepared at different pHs using two buffer systems. The citrate buffer (0.02 N sodium citrate) and phosphate buffer (0.02 N sodium phosphate) containing 0.10 M sodium chloride and 0.01% sodium azide were used from pH 3 to 6 and from pH 6 to 8, respectively. Stock solutions of each buffer were prepared from doubly distilled water. Protein solutions were prepared by adding lyophilized samples to the appropriate volume of buffer. An aliquot (4.0 mL) of protein solution containing 15.0 mg of protein was added to 11.0 mL of buffer in the Petri dish for determination of surface tension. The mixture was gently stirred for 5 s and positioned under the microbalance using a screw-jack platform so that the bottom of the platinum sensor was positioned about 2 mm below the air–protein solution interface when fully lowered. The surface tension of the buffer was 72.0 ± 1.0 mN/M.

The surface tension of the protein solution was continuously monitored after the solution was perfectly still, and the initially formed film was removed by aspiration of the surface. The recorder provided a continuous record of the apparent mass of the sensor, which is proportional to the surface tension. A recorder rate of 2 min/cm was used for the initial 10 min during which γ changed relatively fast, and then a rate of 50 min/cm was used for the next 6 h. As protein adsorbed at the interface, γ decreased, as reflected by the decreasing force on the sensor. The surface tension (γ) of the solution over time was then calculated from the equation $\gamma = F/2L$ where F is the force required to hold the platinum sensor in the interface (Hiemenz, 1977) and L the length (cm) of the platinum sensor. The surface tension of β-LG attained near equilibrium after 6 h. The pH of the protein solution was recorded after each run.

Surface Pressure. The values of γ of β-LG solutions were used to calculate various interfacial properties: surface pressure (Π) changes, rates of adsorption, area cleared per molecule, work of compression during penetration of the protein into the interface, and the apparent number of amino acid residues of β-LG penetrating the interface.

The surface pressure (Π) of a solution corresponds to the reduction of the surface tension by a surfactant (Davies and Rideal, 1963) and is calculated from $\Pi = \gamma_s - \gamma_p$ where γ_s and γ_p are the surface tensions of the buffer and the protein solutions, respectively. The Π of a protein solution increases with time since the surface tension of protein solutions decreases with time. An increased value of Π also corresponds to a decreased amount of free energy at the interface (MacRitchie, 1978).

Rate of Adsorption and Rearrangement of Protein at the Interface. The rate of adsorption and penetration of proteins into the interface can be estimated from changes in Π (Graham and Phillips, 1979a). The number of amino acid residues occupying the interface is indicated by Π; therefore, the rates of penetration of amino acid residues into the interface can be estimated from the rate of change of Π of protein solutions. The number of amino acid residues in the interface may increase by more proteins adsorbing at the interface or by rearrangement of proteins already occupying the interface (Graham and Phillips, 1979a). The rates of these processes can be estimated by the first-order rate equation

$$\ln \frac{\Pi_{ss} - \Pi_t}{\Pi_{ss} - \Pi_0} = -Kt$$

where Π_{ss}, Π_0, and Π_t are the surface pressure values at steady-state conditions, at initial time (t_0), and at time t, respectively, and K is the first-order constant ($K = 1/$relaxation time). A plot of $\ln [(\Pi_{ss} - \Pi_t)/(\Pi_{ss} - \Pi_0)]$ vs. time normally shows two or more linear portions. The initial linear slope corresponds to the first-order rate constant of adsorption (K_1) while the second slope of the plot between 30 and 60 min corresponds to the first-order rate constant of rearrangement (K_2) of the proteins at the interface (Graham and Phillips, 1979a).

Area Cleared and Work of Compression. The values of surface tension over time can be used to determine the area cleared per molecule during adsorption and rearrangement (MacRitchie and Alexander, 1963a; Tornberg, 1978a). As the proteins adsorb at the interface, the molecules already at the interface must be compressed; i.e., an area of the interface must be cleared to allow the newly arrived proteins to penetrate and act as surfactant. The area cleared (dA) before penetration of a protein into the interface and the work of compression (ΠdA) may be estimated from the equation $\ln (d\Pi/dt) = \ln [\Pi(Kc) - (\Pi dA/kT)]$, where K is the first-order rate constant of adsorption or rearrangement, c is the effective concentration of surface-active groups, k is Boltzmann's constant, and T is the absolute temperature. The slope of the plot, $\ln (d\Pi/dt)$ vs. Π, was used to calculate the average area cleared per protein. The values of dA provide an estimation of the size of the protein at the interface in addition to the average area cleared per protein at different surface pressures. A large area of interface needs to be cleared for a whole protein molecule to penetrate the interface, whereas a small area needs to be cleared if only a segment of the protein penetrates into the interface. The apparent number of amino acid residues participating in the adsorption or rearrangement of protein at the interface can be calculated by dividing dA by the average molecular area of amino acid residues (Graham and Phillips, 1979b).

The estimated work required to clear an area at the interface can be calculated from the plot of $\ln (d\Pi/dt)$ vs. Π (MacRitchie and Alexander, 1963a). The amount of work of compression at the interface increases with the concentration of protein at the interface. Since the number of proteins and segments of proteins at the interface increases as the film ages, the amount of work performed by proteins that adsorb or reorient at the interface continually increases with time (MacRitchie, 1978).

RESULTS AND DISCUSSION

Surface Pressure of β-Lactoglobulin. The Π of β-LG solutions increased with time (Figure 1). The rate of increase of Π at pH 3.3 was greater than at pH 5.3 or 6.35. The values of β-LG during the first 10–30 min at pH 3.3 were lower than those of β-LG at pH 5.3 and 6.35, respectively. The rate of increase of Π of at pH 5.3 and 6.35 decreased as the interfacial film aged and reached equilibrium after 360 min at pH 5.3 and 6.35 whereas the Π of β-LG at pH 3.3 had not reached equilibrium by 360 min. These results indicated that the characteristics of the interfacial film of β-LG were affected by the age of the film and the pH of the solution.

The Π reaches an equilibrium value because there is a limit to the number of protein molecules or segments or proteins that can occupy the interface (between 1 and 8 mg of protein/m^2) (MacRitchie, 1978). The length of time required for a protein solution to reach a stable value of Π is a function of the intrinsic characteristics of the protein and the conditions in the system. Native, globular proteins form a more condensed and more tightly packed interfacial film than random-coil or denatured proteins (MacRitchie,

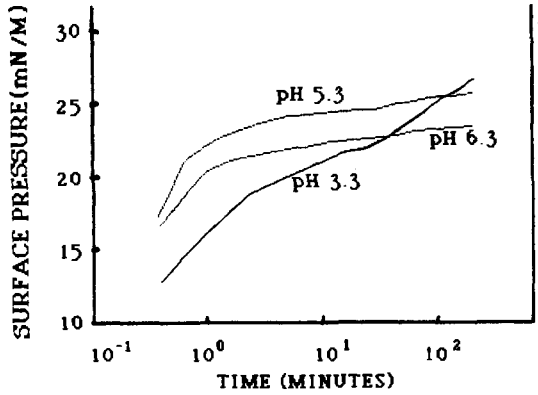

Figure 1. Rate of development of surface pressure of β-lactoglobulin films at the air–water interface at pH 3.3, 5.3, and 6.3.

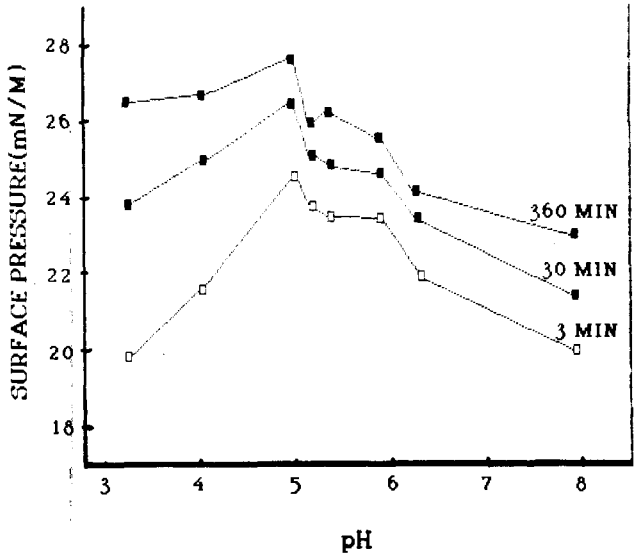

Figure 2. Relationship between pH and surface pressure of β-lactoglobulin films at different time intervals. (See Methods and Materials for details.)

1978). The surface concentration of protein is higher for condensed films than more expanded films because the conformation of the protein at the interface is more compact. Thus, lysozyme, a compact globular protein with a stable tertiary structure, forms a condensed interfacial film because the protein does not denature readily at the interface (Graham and Phillips, 1979c). The surface concentration of lysozyme (10^{-3}–10^{-4}% (w/v)) increases rapidly up to 1 h and subsequently increases slowly because of packing and conformational changes in the protein adsorbed at the interface (MacRitchie, 1978; Graham and Phillips, 1976, 1979b).

The pH of solution greatly affected the rate of adsorption (Figure 2). Thus, Π was significantly greater in the pH range 4.5–6.0 at all sampling periods. This effect was most noticeable at shorter times. Above pH 4.5, Π progressively decreased at all time periods with increasing pH.

Thus, the net charge of the β-LG affected its surface-active properties. The apparent optimum of Π of β-LG occurred at pH 4.9 slightly below the isoelectric point (IEP) of β-LG (IEP = 5.25) (McKenzie, 1971). The rate of adsorption of protein at the interface is increased near the IEP of a protein when the protein remains soluble (Cumper, 1953; MacRitchie, 1978), because the proteins have decreased electrostatic repulsion at the interface and the more compact protein molecules can pack more easily into the interfacial film.

Rates of Adsorption and Rearrangement. The rates

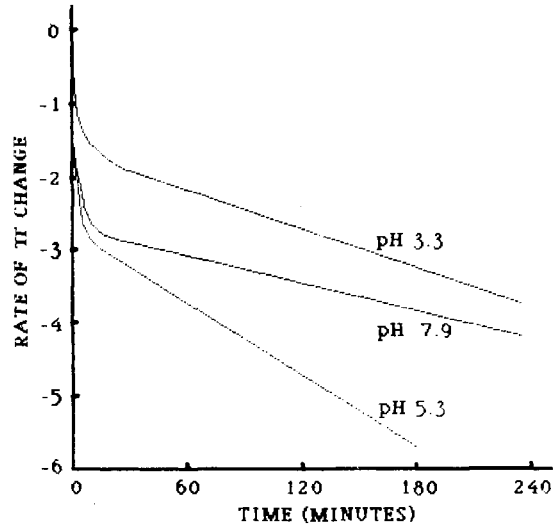

Figure 3. Rate of change of surface pressure $\log_n [(\Pi_{ss} - \Pi_t/\Pi_{ss} - \Pi_0)]$ with aging (time) of β-lactoglobulin films at different pH values. (See text for details.)

of adsorption and rearrangement of molecules of β-LG at the air–water interface were calculated from the values of surface pressure of β-LG at different times and pHs. The increase in Π of a protein over time results from both adsorption and subsequent rearrangement of molecules already at the interface (MacRitchie, 1978; Graham and Phillips, 1979a). Thus, changes in Π provide a measure of the rates of these interactions at the interface. The apparent rate constants of adsorption (K_1) and rearrangement (K_2) of solutions of β-LG were calculated by using the surface pressure at 360 min (Π 360) as the steady-state value and the initial value (Π_0) of β-LG as zero. The values of K_1 and K_2 were estimated from the two linear slopes of the plots of $\ln [(\Pi_{ss} - \Pi_t)/(\Pi_{ss} - \Pi_0)]$ vs. time at different pH values (Figure 3). The K_1 was calculated from the initial slope of the graph, i.e. during the first few minutes, while K_2 was calculated from the slope of the graph from 60 to 180 min. The relaxation times of adsorption and rearrangement were less than 5 min and several hours, respectively (relaxation time $1/K$).

The effects of pH on K_1 and K_2 of β-LG are summarized in Figure 4, parts A and B, respectively. The more rapid rates of adsorption and rearrangement corresponds to the larger numbers, i.e., the values of K_1 and K_2 of β-LG were highest between pH 4.5 and 6.5 and pH 4.5 and 5.5, respectively. The highest rates of rearrangement of β-LG were observed when the protein had the lowest net charge, close to its isoelectric point (IEP). These data indicate that the rate of Π development at a different pH (Figure 2) may reflect the rate of rearrangement more than adsorption.

Area Cleared Per Protein and the Work of Compression. Information about the molecular interactions of β-LG at the air–water interface was derived from the changes in Π with time. These analyses provide estimates of the area of the interface that must be cleared for the penetration of a molecule of β-LG (dA) and the work of compression required to clear an area at the interface (ΠdA) for the new protein (MacRitchie, 1978; Tornberg, 1978a). The assumptions used for these calculations were that the protein was irreversibly adsorbed at the interface, the kinetics of the adsorption and rearrangement depended upon one component (first-order kinetics), the activity of the protein in solution was equal to its concentration, and the change in the surface pressure (dΠ) was proportional and nearly equal to the change in the number of proteins or segments of proteins at the air–water interface

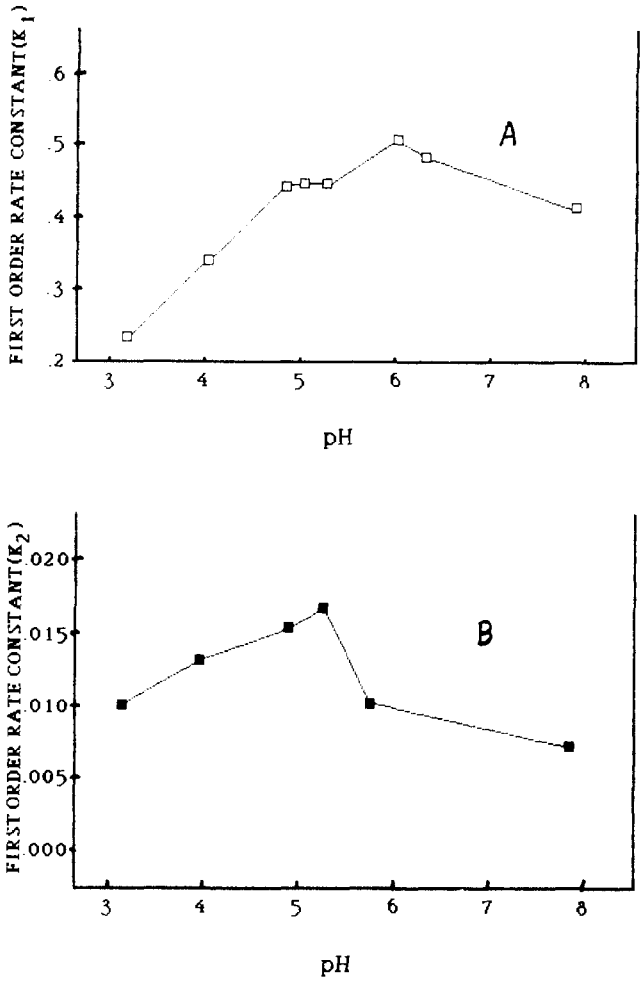

Figure 4. Effect of pH on the first-order rate constants of (A) adsorption (K_1) (1/min) and of (B) rearrangement (K_2) of β-lactoglobulin at the air–water interface. The first-order rate constants were determined by using the initial slope of the plot of surface concentration vs. time. (Figure 3) (See Methods and Materials for details.)

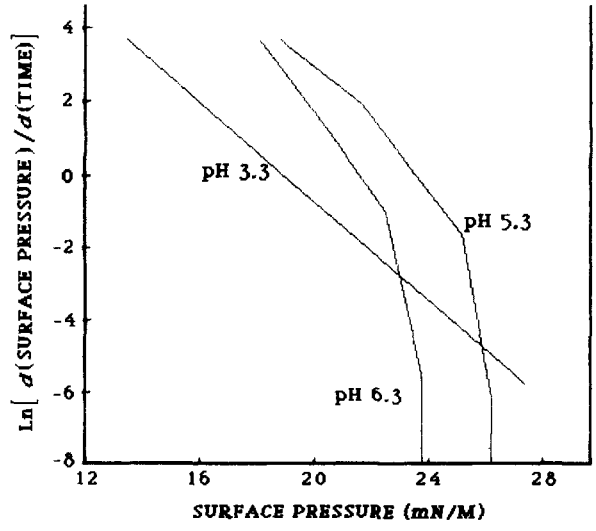

Figure 5. Effect of surface pressure on the rate of change of surface pressure (dΠ/dt) of β-lactoglobulin at pH 3.3, 5.3, and 6.35. (See Methods and Materials for details.)

(MacRitchie, 1978; Graham and Phillips, 1979b).

The values of dA and ΠdA of β-LG were calculated from the slope(s) of the plots of ln (dΠ/dt) vs. Π (Figure 5). The slopes of the lines varied with pH treatments, possibly reflecting the different molecular processes occurring

Table I. Average Area Cleared Per Protein Molecule during Adsorption and Rearrangement and Apparent Number of Amino Acid Residues Penetrating the Air–Water Interface at Progressive Periods of Time during Film Formation by β-Lactoglobulin

treatment (pH)	time period, min	av area cleared per protein, nm^2	app no. of amino acid res penetrating interface
3.29	0.4–360	2.6	17.3
4.06	0.8–8	2.9	19.3
4.06	6–120	4.5	30.0
4.06	60–360	20.6	137.3
4.87	0.4–3	3.5	25.3
4.87	2–60	6.4	42.7
5.05	0.4–10	5.1	34.0
5.05	6–360	13.1	87.3
5.85	0.4–6	4.8	32.0
5.85	6–360	11.9	79.3
6.35	0.4–3	4.7	31.3
6.36	2–60	7.7	51.3

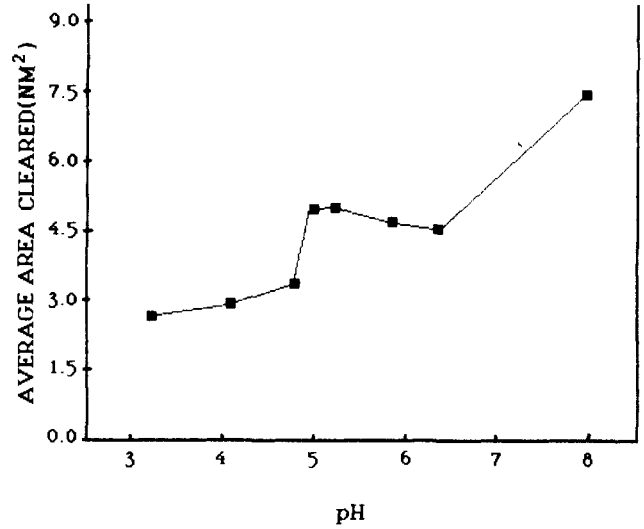

Figure 6. Effect of pH on the average area cleared per protein during the adsorption of β-lactoglobulin at the air–water interface. (See Methods and Materials for details.)

during the development of surface pressure of β-LG. The initial increase in β-LG (0–15 mN/M) was not recorded because it took 15 s to take the first reliable measurement.

The process of rearrangement at the interface begins when the first few molecules of protein arrive at the interface, and it continues until equilibrium is attained (MacRitchie, 1978). The process of rearrangement of molecules of protein at the interface depends upon the conformational stability of the protein and the specific chemical forces acting on the proteins at the interface. Adsorption decreases considerably before the surface pressure of the protein solution reaches equilibrium (Graham and Phillips, 1979b). Thus, rearrangement of protein may contribute more to changes in the surface pressure after 60 min than adsorption of new protein at the interface. Therefore, the first slope (Figure 5) may correspond primarily to changes in Π arising from the process of adsorption while the second slope corresponds mostly to changes resulting from rearrangement of molecules at the interface. The slopes generally increased with time. The steeper slope indicated that penetration and rearrangement of β-LG continued, but at a rate slower than that of the initial adsorption which is a diffusion controlled process and is consistent with the results of MacRitchie and Alexander (1963a) and Tornberg (1978a,b).

The effects of pH on dA dn ΠdA of β-LG are shown in Figure 6 and Tables I and II. The values of dA of β-LG

Table II. Work of Compression (ΠdA) Performed by Molecules of β-Lactoglobulin While Penetrating the Film of Protein at the Air-Water Interface after Adsorption for 3 and 30 min

	work of compressn ($\times 10^{14}$ erg)	
treatment (pH)	3 min	30 min
3.29	48.9	59.5
4.06	63.2	111.6
4.87	85.4	168.3
5.05	120.0	327.9
5.85	118.3	1133.7
6.35	113.3	177.9
7.29	150.0	160.5

(Figure 6) correspond to the values calculated from the first slope of the graph (Figure 5), i.e. from the process of adsorption. All values of dA for β-LG are tabulated in Table I. The apparent work performed by proteins (ΠdA) in compressing the interface during the different processes was calculated for two periods: 3 and 30 min (Table II).

The values of dA and ΠdA, corresponding to adsorption and rearrangement of LG increased as the surface pressure of the interface increased, i.e. as the film aged (Tables I and II). The values of ΠdA and dA, especially ΠdA at 3 min, increased as the pH was increased (Figure 6; Table II).

The increased values of dA and ΠdA of β-LG could result from a more condensed interfacial film, from greater electrostatic interactions of proteins at the interface, or from an increased amount of dehydration during penetration (Tornberg, 1978a; MacRitchie, 1978; Ter-Minassian-Saraga, 1981). The values of dA and ΠdA of β-LG near pH 5.3 were probably increased because the molecules of β-LG formed a more condensed film near the IEP while the increased values of dA and ΠdA at higher pHs were probably caused by the increased electrostatic repulsion between the proteins and increased hydration of charged groups. The maximum area that needed to be cleared for the adsorption of a molecule of β-LG occurred at pH 8.0. This was also the pH of maximum work of compression during the adsorption process (Table II). Apparently increased electrostatic repulsion between proteins at the interface increased the amount of energy required to penetrate the film.

The area cleared per protein during adsorption of β-LG was slightly larger near its IEP than at lower or slightly higher pHs. This may reflect the greater amount of protein at the interface since the rate of adsorption and the Π of β-LG was increased near its IEP. A slightly larger value of the work of compression of β-LG was also observed near the IEP probably for the same reasons.

MacRitchie and Alexander (1963a) reported an area of 1–2 nm^2 per protein during the adsorption of BSA from a 0.003% solution. Graham and Phillips (1979a) reported the dA of BSA and lysozyme as 0.5 and 2.4 nm^2, respectively, at protein concentrations of about 0.0001%. Tornberg (1978b) observed that dA of proteins increased with substrate concentration and sodium chloride. The values of dA for adsorption and rearrangement were 1–7 nm^2 for soy protein, 2–16 nm^2 for casein, and 1–7 nm^2 for whey protein at a substrate concentration of 0.1% (pH 7). Thus, the values of dA of β-LG, i.e. 2–115 nm^2, were higher than for whey protein concentrate reported by Tornberg (1978b). The values of dA corresponding to adsorption of β-LG were similar to the literature values, but the values corresponding to rearrangement were higher (MacRitchie, 1978; Graham and Phillips, 1979).

The number of amino acid residues of β-LG that penetrated the interface was estimated by dividing dA by 0.15 nm^2 per amino acid residue (Graham and Phillips, 1979c). Since β-LG has only 161 amino acid residues (McKenzie, 1971), values of dA greater than 24 should not be observed according to this equation. High values of dA probably arose because assumptions made did not hold in all cases. The value of $d\Pi/dt$ probably did not correspond to the value of dn/dt, i.e. the change in the number of residues penetrating the interface, at all times especially in the initial period. The number of amino acid residues of β-LG penetrating the interface depended upon the age of the interfacial film and the pH (Table I). The number of amino acid residues of β-LG penetrating the interface increased with time. This was expected since the values of dA increased with time. The size of the area cleared during adsorption of β-LG was between 17 and 51 amino acid residues or 10–32% of the amino acid residues of β-LG. This indicated that the tertiary structure of β-LG was probably not altered very much before it penetrated the interface. The number of amino acid residues of β-LG rearranging at the interface ranged from 17 to 137 residues (normally around 40–80 residues) (Table I). Thus, more amino acid residues were involved in conformational changes of β-LG during rearrangement at the interface than during the penetration of the protein at the interface.

The high values of dA corresponding to rearrangement of β-LG indicated that a large portion of the β-LG molecule was involved in the rearrangement processes and/or the amount of dehydration of the protein was large. Both phenomena could arise from a condensed interfacial film where the amount of kinetic energy required to alter the structure of the membrane is large.

These data indicate that the properties of protein films are significantly affected by aging of film and by electrostatic phenomena. These reflect the importance of protein–protein interactions in forming the film network structure. The magnitudes of these are critical in determining the whipping/foaming properties of food proteins (Kinsella, 1981; German et al., 1985; Kim and Kinsella, 1985; Graham and Phillips, 1976).

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Thermal Acid-Catalyzed Rearrangement of *trans*-Methyl Chrysanthemate to Lavandulyl Derivatives and Their Effect on Lettuce Seedling Growth

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Acid-catalyzed rearrangement of *trans*-methyl chrysanthemate in aqueous sulfuric acid gave only lavandulyl methyl esters at room temperature and a mixture of unsaturated γ- and δ-lactones at 130 °C. Acid-catalyzed methanolysis afforded the methoxylavandulyl methyl ester and its isomer methyl 2,2-dimethyl-3-(2-methoxy-2-methylpropyl)cyclopropanecarboxylate. The starting material was obtained from a mixture of *cis*/*trans*-chrysanthemic acid by lactonizing the cis isomer to *cis*-dihydrochrysanthemo-δ-lactone which was separated and then esterifying the trans chrysanthemic acid. It was found that the δ-lactone, certain lavandulyl methyl esters, and certain unsaturated γ- and δ-lactones displayed potent inhibitory effect on root elongation and lettuce seed germination at 10^{-4} and 10^{-5} M concentrations. The relation of structure and lettuce seedling growth inhibitory activity is discussed. Structural determination of the saturated δ-lactone was carried out by single-crystal X-ray diffraction analysis.

The cyclopropane monoterpene *trans*-chrysanthemic acid [**3**] is found in three of the six natural insecticidal esters (Pyrethrin I, Cinerin I, Jasmolin I) of the pyrethrum flower *Chrysanthemum cinerariaefolim*. During our investigation of the acid-catalyzed reactions of *trans*-methyl chrysanthemate [**5**] (Goldschmidt et al., 1984b) obtained by known procedures from readily available *cis*/*trans*-ethyl chrysanthemate [**1**], it was found that three principal methyl lavandulyl esters were obtained when **5** was stirred with 50% aqueous sulfuric acid and pentane at room temperature. Spectral and microanalytical data confirmed that these esters were methyl *trans*-5-methyl-2-(2-propenyl)-3-hexenoate [**7**], methyl *trans*-5-methyl-2-(2-hydroxy-2-propyl)-3-hexenoate [**8**], and methyl *trans*-5-methyl-2-(2-propylidene)-3-hexenoate [**10**]. The methoxy lavandulyl ester methyl *trans*-5-methyl-2-(2-methoxy-2-propyl)-3-hexenoate [**9**] was detected in low yield but was obtained in good yield from **5** and 50% methanolic sulfuric acid in pentane at room temperature. The isomer of **9**, 2,2-dimethyl-3-(2-methoxy-2-methylpropyl)cyclopropanecarboxylate [**6**], was also isolated from the reaction. When *trans*-methyl chrysanthemate [**5**] was stirred with a mixture of 50% aqueous sulfuric acid in hexane at 130 °C for 44 h there was obtained the principal γ-lactone, dihydro-5-(2-propyl)-3-(2-propylidene)-2(3*H*)-furanone [**12**], and the δ-lactone, 5,6-dihydro-6,6-dimethyl-3-(2-propyl)-2*H*-pyran-2-one [**11**] (Figure 1).

During the last decade certain chrysanthemic acid esters were found to possess plant growth regulatory activity. It was reported that *cis*/*trans*-2-(*N*,*N*,*N*-trimethylamino)-ethyl chrysanthemate chloride caused stunting in corn, milo, and cotton plants (Faucher, 1974). The synthetically insecticidally active pyrethroid decamethrin was found to inhibit the gibberellin-stimulated growth of rice (Herve and Motillon, 1981).

MATERIALS AND METHODS

Spectroscopy. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker WX 300 spectrometer. Infrared (IR) spectra were recorded on a Perkin-Elmer

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