STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LIII, 1, 2008

In memoriam prof. dr. Liviu Oniciu

KINETIC AND THERMODYNAMIC CHARACTERIZATION OF PROTEIN ADSORPTION AT FLUID INTERFACES

MARIA TOMOAIA-COTISEL[a], OSSI HOROVITZ[a], OLIMPIA BOROSTEAN[a], LIVIU-DOREL BOBOS[a], GHEORGHE TOMOAIA[b], AURORA MOCANU[a] AND TRAIANOS YUPSANIS[c]

ABSTRACT. The formation and characterization of nanostructured polyfunctional layers (films) based on protein adsorption at different fluid interfaces, such as air/water or oil/water interfaces, in the absence or in the presence of stearic acid are investigated. For instance, kinetics and thermodynamics of protein adsorption at the air/aqueous solutions were studied, thereby evidencing the protein surface active properties. The investigated protein was a globulin extracted and purified from aleurone cells of barley. The conjugated effect of protein and stearic acid simultaneous adsorption was also investigated, at the benzene/aqueous solutions interface. A stable mixed lipid and protein film has been formed by the co-adsorption of these biomolecules at liquid-liquid interface showing that the interaction between stearic acid and the protein is significant.

Keywords: *adsorption, fluid interfaces, protein, stearic acid*

INTRODUCTION

A great number of problems in interface science deal with the adsorption [1-15] and relaxation [5, 16-20] of surface active compounds (in short, surfactants) at fluid interfaces. In fact, the modern soft-matter physical chemistry has opened a great number of questions dealing with the dynamics of soft surfaces, particularly with protein or lipid dynamics at interfaces. Among these systems, adsorbed protein films are frequently considered as model systems to explore the surface behavior of proteins or their interaction with lipids at fluid interfaces. In vivo, the control over protein functional behavior is often mediated by the formation of supramolecular assemblies with lipids that frequently play a crucial role in the molecular organization of biological systems.

[a] *Babes-Bolyai University of Cluj-Napoca, Faculty of Chemistry and Chemical Engineering, Arany J. Str., no 11, 400028 Cluj-Napoca, Romania, mcotisel@chem.ubbcluj.ro*

[b] *Iuliu Hatieganu University of Medicine and Pharmacy, Department of Orthopedic Surgery, Mosoiu Str., no. 47, 400132 Cluj-Napoca, Romania*

[c] *Aristotelian University, School of Chemistry, Laboratory of Biochemistry, 54006 Thessaloniki, Greece*

Protein adsorption at interfaces is also an important subject of investigations, in many artificial systems encountered in industrial applications, particularly because many alimentary emulsions are stabilized by proteins. The understanding of protein behavior and its interactions with other biomolecules might bring strong information for a desired performance.

Usually protein adsorption at fluid interfaces is studied by measurements of the interfacial tension between the two phases (*e.g.,* oil and water) [21-23]. By protein adsorption, the interfacial tension is reduced in time, for a particular concentration in protein, and as a function of protein concentration. From interfacial tension isotherms, information is gained upon the processes taking place in the early stage of adsorption [24]. Generally, the interfacial phenomena and adsorption kinetics are essential in determining chemical and physical properties of such systems [25]. Thus, the adsorption kinetics of egg yolk was studied at the triacylglycerol / water interface and the effect of pH was assessed [26]. The role of the charged protein surface in the adsorption dynamics was studied on L-glutamic acid copolymers [27].

Model for protein adsorption at interfaces

Experimentally, it was found that the dynamics of surface tension presents three regimes [24], specific for diluted solutions of different proteins (Fig. 1):

- an induction regime, noted regime I, where the interfacial tension remains relatively constant, at the values characteristic for the pure liquid phase (e.g., the air/water interface)
- the second regime, noted regime II, is distinguished by a sudden drop of interfacial tension from its initial value
- the last regime, named regime III, corresponds to a quasi-linear decrease of interfacial tension, with a less abrupt slope than for the second regime.

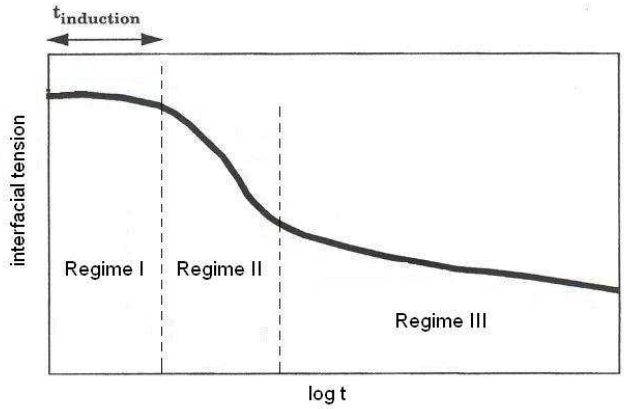

Figure 1. Plot in semilogarithmic coordinates corresponding to the three adsorption regimes of proteins at fluid interfaces.

An induction time, as in the first regime, is frequently observed in protein solutions at low concentrations, at the air/water interface [28, 29]. Protein molecules are present at the interface, but they do not reduce considerably the interfacial tension. In this period, the diffusion of protein molecules to the surface is important in controlling the adsorption, and is followed by modifications of the protein configuration (denaturation). An induction period is observed only for much diluted protein solutions. In more concentrated solutions, regime III is directly attained.

In the regime II, there is already a saturated protein monolayer at the interface, but the relaxation in the conformation of proteins makes possible both the adsorption of more inner segments, of the protein molecules at the interface, and the diffusion of more protein molecules from the bulk to the interface. Both effects contribute to the decrease of interfacial tension in time. Conformational modifications and denaturation of proteins are therefore more important in reducing interfacial tension than the initial diffusion and adsorption of protein molecules. It is recognized that the protein adsorption corresponding to the regime II can give indications on the protein conformational stability of adsorbed proteins.

In the regime III, there is only a slight decrease of interfacial tension, ascribed to conformational modifications in the adsorption layer with the formation of multilayers and consequently building of o continuous protein gel lattice at the interface.

In many cases, the time dependence of interfacial tension is a logarithmic one, $\sigma(t) \sim k \log t$, for the protein adsorption at the air/water interface.

Among proteins, the plant proteins are particularly important because they are largely used as ingredients in human alimentation and in other fields, such as cosmetics or drugs delivery systems. Adsorption properties of plant proteins (such as α-gliadins or pea protein) were investigated at the oil / water interface [30] and compared with the corresponding properties of gelatin.

Recently, we studied the adsorption of a plant protein, namely the major globular protein extracted from aleurone cells of barley [31], on various surfaces, such as glass or mica [32, 33], the surface of citrate anions capped gold nanoparticles in colloidal aqueous solutions [34, 35], and on gold nanoparticles auto-assembled as an interfacial film on a solid surface [36], using UV-Vis spectroscopy, TEM and AFM observations.

In the present paper we investigate the adsorption of the same globular protein at fluid interfaces, such as the air/water and oil/water interfaces, as well as the simultaneous adsorption of the protein and stearic acid at the interface between the aqueous and benzene solutions.

RESULTS AND DISCUSSION

Protein adsorption at the air/water interface

Table 1.

Interfacial tension, σ, at the air/aqueous 0.5 M NaCl solution interface, at 20 ^{o}C, for different bulk protein concentrations, C_p, in the aqueous phase, at different times, t, of adsorption

C_p = 5 mg/L		C_p = 4 mg/L		C_p = 3 mg/L		C_p = 2 mg/L		C_p = 1 mg/L		C_p = 0.5 mg/L	
t, min	σ, mN/m	t, min	σ, mN/m	t, min	σ, mN/m	t, min	σ, mN/m	t, min	σ, mN/m	t, min	σ, mN/m
0	67.77	0	72.3	0	72.25	0	72.07	0	72.19	0	72.13
5	66.51	5	71.85	5	72.08	5	72.02	5	72.11	5	72.10
10	65.8	10	71.01	10	71.99	10	71.87	10	72.08	10	72.08
15	65.3	15	70.52	15	71.91	15	71.73	15	72.02	15	72.05
20	64.93	20	70.01	20	71.65	20	71.62	20	71.99	20	72.02
25	64.73	25	69.32	25	71.59	25	71.50	25	71.93	25	72.02
30	64.44	30	69.09	30	71.42	30	71.39	30	71.88	30	71.99
40	64.16	40	68.45	40	70.98	40	71.10	40	71.82	40	71.96
50	63.59	50	67.99	50	70.69	50	70.87	50	71.73	50	71.93
60	63.33	60	67.48	60	70.06	60	70.55	60	71.59	60	71.90
80	62.84	80	66.84	80	69.25	80	69.92	80	71.36	80	71.87
100	62.66	100	65.98	100	68.74	100	69.32	100	71.16	100	71.85
120	62.56	120	65.35	120	67.99	120	69.11	120	70.93	120	71.79
150	62.44	150	64.31	150	67.12	150	68.34	150	70.44	150	71.73
180	62.32	180	63.85	180	66.31	180	67.76	180	70.06	180	71.64
210	62.26	210	63.39	210	65.97	210	67.30	210	69.69	210	71.50
240	62.26	240	63.28	240	65.45	240	66.96	240	69.49	240	71.39
		270	63.05	270	65.03	270	66.44	270	69.28	270	71.30
		300	62.97	300	64.93	300	66.27	300	68.94	300	71.13
		330	62.87	330	64.73	330	66.12	330	68.77	330	70.93
		360	62.83	360	64.56	360	65.81	360	68.54	360	70.75
		410	62.79	390	64.38	390	65.64	390	68.48	390	70.70
		450	62.8	420	64.15	420	65.26	480	68.23	420	70.61
		480	62.65	450	64.01	450	65.06	570	68.06	450	70.58
		510	62.56	480	63.81	480	65.06	600	68.02	480	70.55
		540	62.56	510	63.69	510	65.03			510	70.52
		570	62.64	540	63.60	540	64.97			540	70.52
				570	63.43	570	64.78				
						600	64.60				
						630	64.51				

The values of the interfacial tension measured at different times of protein adsorption, for each of the investigated protein concentrations, at the air/aqueous (0.5 M NaCl) solution interface, are given in Table 1.

From this table, it can be observed that the interfacial tension varies strongly with time, the adsorption equilibrium being reached in more than 10 hours for some protein concentrations. The equilibrium is indicated by the almost constant value of the interfacial tension.

Further, using semilogarithmic coordinates, the representation of interfacial tension against the logarithm of the adsorption time, $\sigma = f(\log t)$, is given in Fig. 2. The general aspect of the plots is similar to that resulting from the model of the three adsorption regimes of proteins (Fig.1). While regime I (induction period) and II (monolayer saturation) are clearly evidenced in the plots, for regime III the beginning is barely outlined for the highest concentration (5 mg/L). On the other hand, the induction period is clearly delimited only for the lower protein concentrations (below 4 mg/L).

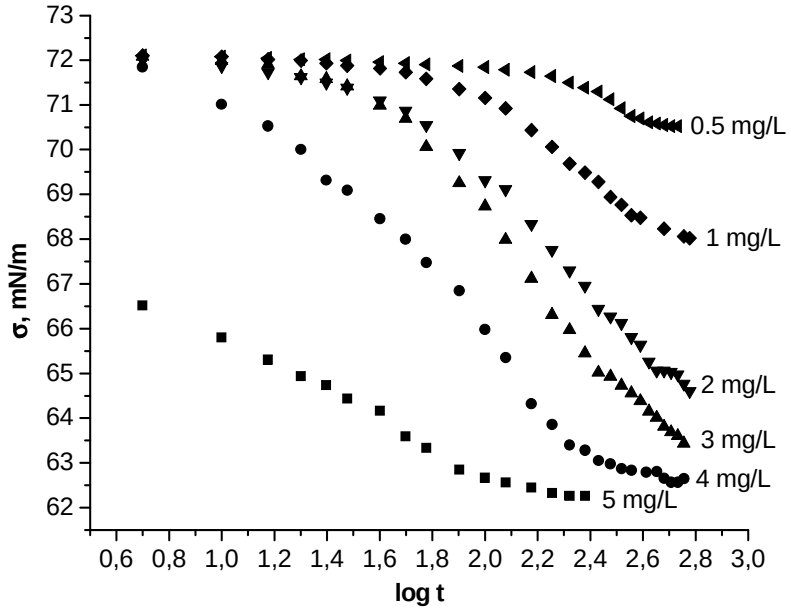

Figure 2. Representation in semilogarithmic coordinates of dynamic interfacial tension (mN/m) against time (min) for protein adsorption from the aqueous phase (with various protein concentrations), at the interface with air, at a temperature of 20 ^{o}C.

The limiting values of the interfacial tensions, those corresponding to the maximum time of protein adsorption for each protein concentration can be considered for the establishing of thermodynamic adsorption equilibrium that is given by the static interfacial tension. The representation of these static interfacial tensions versus C_p concentration (Figure 3) shows the typical appearance of a surface tension isotherm, in presence of a surface active substance. The surface activity of the protein at the air / aqueous solution interface is thus confirmed.

The adsorption equilibrium and the final adsorption regime III occur upon protein adsorbed monolayer coverage, and is attributed to continued relaxation of the adsorbed layer and possible build-up of protein multilayers, as depicted in Figure 4.

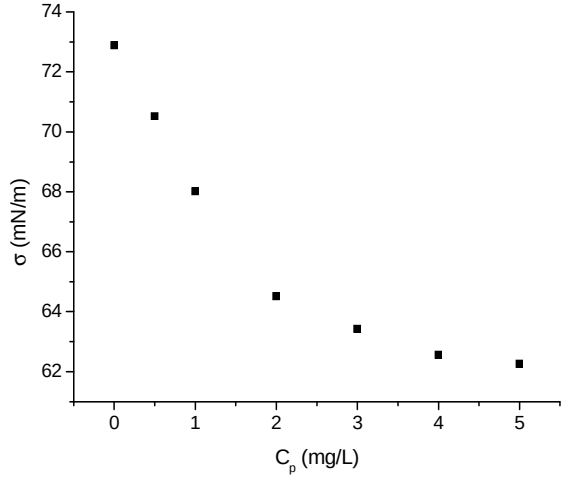

Figure 3. Variation of the static interfacial tension at the interface with air, for protein adsorption from the aqueous phase, against the protein concentration at 20 ^{o}C temperature.

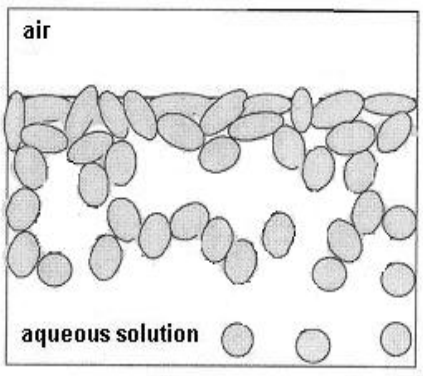

Figure 4. Schematic representation of protein adsorption at interface (regime III)

Protein and stearic acid co-adsorption at the oil/water interface

Furthermore, we studied the lipid and protein layers at the benzene/ aqueous (0.5 M NaCl) solutions interface. To explore the interaction between lipid and protein, we have chosen a fatty acid (stearic acid) as a simple

model for lipids. For the beginning we studied the adsorption of protein and separately the adsorption of stearic acid at the same interface oil/water for two distinct temperatures, 20 and 36 ^{O}C.

The dynamics of protein adsorption at benzene/water (0.5 M NaCl) interface is examined over the time scales ranging from seconds to several hours by measuring the interfacial tension at the benzene/aqueous solutions interface for the chosen constant temperature. The adsorption of protein at the benzene/aqueous solution interface leads to an interfacial tension versus time profile that presents common characteristics with the adsorption of bovine serum album at the benzene/water interface [40, 41]. For oil/water interface the induction period for protein adsorption was not detected. The adsorption equilibrium of protein appears to be established in about 60 min at the oil/water interface. The equilibrium data for pure protein adsorbed at the benzene/aqueous interface are given in Tables 2 and 3 at 20 and 36 ^{O}C, respectively.

The protein diffusion and protein interfacial affinity determine the duration of early stages of adsorption period at fluid interfaces. Continued protein rearrangement leads to the final interfacial tension reduction resulting in various interfacial contacts per protein molecule.

Independently, the adsorption of stearic acid at the benzene/aqueous (0.5 M NaCl, pH 5.5) solution interface was investigated. The adsorption behavior of stearic acid is similar with its adsorption at the interface benzene/ water (pH 2) interface. At pH 2, stearic acid (pK_a = 5.63 [42] is neutral and at pH 5.5 it is almost 50% ionized. The static adsorption is reached at about 100 min depending on the stearic acid bulk concentration in substantial agreement with data published by us earlier [39]. The equilibrium data for the adsorption of stearic acid at benzene/water interface are given in Table 2 (for 20 ^{o}C) and in Table 3 (for 36 ^{o}C).

For co-adsorption of stearic acid and protein at benzene/water interface it was observed that the adsorption equilibrium is apparently reached in about 60 min. Therefore, for mixed layers of protein and stearic acid, the equilibrium (static) interfacial intension is recorded at an adsorption time of 60 min. It is to be mentioned that for very long adsorption times (several hours) some aging effects are noticed.

For comparison, the co-adsorption of protein and stearic acid at the oil/water interface was recorded by interfacial tension measurements executed at 60 min after the oil/water interface was formed, for both temperatures investigated, 20 and 36^{o}C. The results are also given in Tables 2 and 3.

To avoid the superposition of a strongly time dependent effect, due to the protein adsorption over the one generated by the adsorption of stearic acid from the benzene phase, we have chosen the 2.9 mg/L concentration of protein in the aqueous solutions. The concentration of stearic acid was varied in the range of 0.025 to 0.4 M stearic acid in benzene.

Table 2.

Interfacial tensions (σ) and interfacial pressures (Π) for the simultaneous adsorption of stearic acid (SA) and protein (P) at the benzene/aqueous solution interface, at a constant temperature of 20 ^{o}C.

	σ (mN/m)		Π (mN/m)	
C_{SA} (mol/L)	C_p = 0	C_p = 2.9 mg/L	SA	P
1	2	3	4	5
0.000	34.70	24.90	0.00	9.80
0.030	29.25	24.40	5.45	4.85
0.039	28.43	23.64	6.27	4.79
0.050	27.24	22.58	7.46	4.66
0.064	25.25	21.82	9.45	3.43
0.082	23.82	21.23	10.88	2.59
0.105	22.13	20.14	12.57	1.99
0.136	20.49	19.32	14.21	1.17
0.174	19.15	18.20	15.55	0.95
0.223	17.57	17.15	17.13	0.42
0.287	16.60	16.28	18.10	0.32
0.368	15.81	15.51	18.89	0.30

Table 3.

Interfacial tensions (σ) and interfacial pressures (Π) for the simultaneous adsorption of stearic acid (SA) and protein (P) at the benzene/aqueous solution interface, at constant temperature of 36 ^{o}C.

	σ (mN/m)		Π (mN/m)	
C_{SA} (mol/L)	C_p = 0	C_p = 2.9 mg/L	SA	P
1	2	3	4	5
0.000	35.10	25.17	0.00	9.93
0.025	31.22	22.31	3.88	8.91
0.034	30.05	21.91	5.05	8.14
0.040	29.80	21.63	5.30	8.17
0.050	29.47	21.47	5.63	8.00
0.065	28.78	20.93	6.32	7.85
0.083	28.20	20.44	6.90	7.76
0.109	26.88	19.58	8.22	7.30
0.135	25.84	18.88	9.26	6.96
0.176	24.74	18.59	10.36	6.15
0.225	23.92	17.78	11.18	6.14
0.287	22.60	17.14	12.50	5.46
0.368	20.98	16.13	14.12	4.85

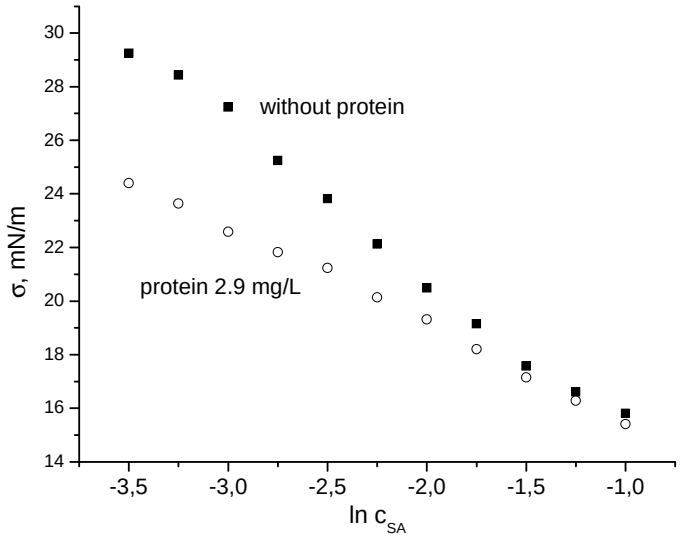

Figure 5. Equilibrium interfacial tension, σ, against the logarithm of stearic acid molar concentration, c_{SA}, in the organic phase, for the interface aqueous 0.5 M NaCl solution / benzene, in absence and presence of protein in the aqueous phase at 20 ^{o}C

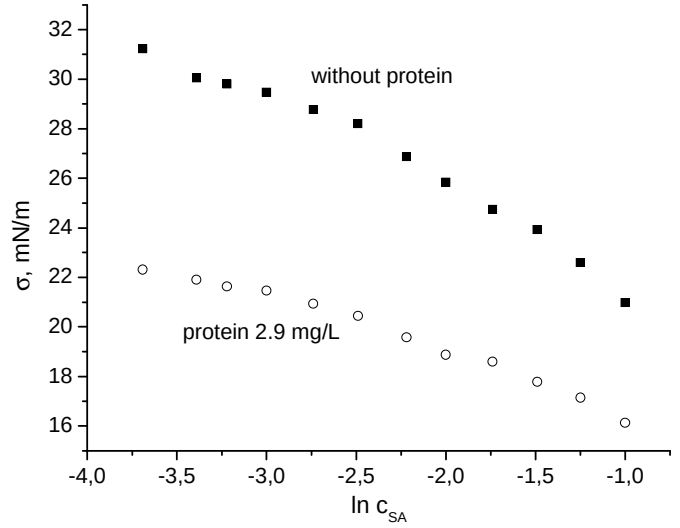

Figure 6. Equilibrium interfacial tension, σ, against the logarithm of stearic acid molar concentration, c_{SA}, in the organic phase, for the interface aqueous 0.5 M NaCl solution / benzene, in absence and presence of protein in the aqueous phase at 36 ^{o}C.

In Figs 5 and 6 the static interfacial tension isotherms versus stearic acid concentration (C_{SA}) are given for the situation without and with protein (2.9 mg/L concentration) in aqueous solutions. From the adsorption isotherm, it is to be noted that the stearic acid is surface active at the benzene/water interfaces.

The processing of the isotherms according to Gibbs equation:

$$\left(\frac{\partial\sigma}{\partial \ln c_{SA}}\right)_T = -\mathrm{R}T\Gamma$$

allows for the determination of the SA surface concentration (Γ) for a certain interfacial tension and the corresponding molecular area: $A = \frac{1}{\Gamma N_A}$, N_A being the Avogadro's constant; R and T having their usual meanings. For the saturation of the interface with adsorbed SA, the limiting molecular areas are determined for stearic acid adsorbed at the oil/water interface with or without protein at two temperatures, 20 and 36 ^{o}C, see Table 4.

Table 4.

Limiting molecular areas, A_0, and the correlation coefficients, r, for adsorbed stearic acid film at the benzene/water interfaces, in the absence and the presence of protein, for two temperatures.

Adsorbed layers	20 ^{o}C		36 ^{o}C	
	A_0, Å^2/molecule	Standard error	A_0, Å^2/molecule	Standard error
SA	79.6	±4.9	87.0	±4.7
SA and protein	111.5	±1.8	124	±7

The comparison of the limiting molecular areas for stearic acid in the absence of protein leads us to the conclusion that the SA film at the benzene/water interface is more expanded than the pure SA film at the air/water interface, A_o = 20 Å^2 [39]. The area increase in adsorbed SA as compared to spread film is due to the benzene molecules which penetrate between the hydrocarbon chains of the SA interfacial film, tending to reduce the attraction among the SA chains.

Also, the limiting molecular areas, A_0, for stearic acid in the presence of protein is much greater than the area of pure SA oriented at one and the same interface at constant temperature. The protein effect is related with the penetration of protein among the hydrocarbon chains of SA film adsorbed at the oil/water interfaces. The increase in temperature brings also an expanding effect upon SA adsorbed film at benzene/water interface.

The equilibrium interfacial tensions are apparently established in about 60 min in contrast with 100 min for pure stearic acid adsorbed film at the oil/water interfaces. The more rapid attainment of the adsorption equilibrium

of SA at the benzene/water interface in the presence of protein is probably due to the interaction between SA and protein in the interfacial adsorbed mixed film.

In order to get a better image of the possible interactions in the mixed SA and P film, data regarding the variation of the equilibrium interfacial tension with SA concentration in the absence (column 2) and in the presence of protein (column 3) in Tables 3 and 4, for 20 and 36 ^{o}C, respectively were compared. It is evident that the presence of protein leads to an additional decrease of interfacial tension, respectively to an increase of the pressure in the adsorbed SA film for all concentrations of SA. Column 4 lists the interfacial pressures of pure SA. The last column shows the contribution of protein to the interfacial pressure of the mixed SA and P film, the contributions of SA (column 4) being assumed as constant. The contribution of protein was evaluated from the difference of the values in columns 2 and 3 for each individual concentration of SA. It is noted that the interfacial pressure due to the protein decreases with the increasing of SA concentration. In other words, the contribution of each component to the interfacial pressure of the mixed adsorbed film is not independent. This fact also suggests the interaction between SA and protein.

Similar cases are reported in the literature on mixed lipid and protein films obtained by penetration of lipid monolayers spread at the air/water interface by an injected protein in the aqueous phase under the lipid monolayer. The penetration of the protein in the lipid layer leads to an increase of its surface pressure at constant area, and the pressure increment was considered a measure of the lipid and protein interaction [43]. This situation corresponds to a sequentially adsorbed lipid and protein mixed film.

In our case, in the complex process of adsorption and penetration of SA and protein at the oil/water interface, the interaction between the stearic acid and the protein is revealed by the decrease of interfacial tension, respectively the increase of the interfacial pressure at the benzene/water interfaces. The increment of interfacial pressure is attributed to the mutual penetration of the two adsorbed films. The interaction between carboxyl groups or the negatively charged carboxylate of SA molecules and the peptide bridges of protein or with protein positively charged regions may be suggested, as well as the interaction among their hydrocarbon chains, which is not to be completely neglected even at the oil/water interface.

Moreover, in our case, the simultaneously adsorbed stearic acid and protein mixed films are formed where the molecular associations can be generated at the interfaces, such as negatively charged complexes when stearate molecules (negatively charged at pH 5.5) are involved in associations and neutral complexes when stearic acid not charged is involved in the interaction within mixed adsorbed layers at oil/water interfaces, as recently emphasized for β-lactoglobulin and pectin in adsorbed layers at fluid interfaces [2].

CONCLUSIONS

Our previous studies on the adsorption of the globular storage protein, extracted and purified from aleurone cells of barley, at the solid interface of gold nanoparticles is completed by the investigation of the adsorption of the same protein at gas/liquid and liquid/liquid interfaces. The evolution in time of the protein adsorption at the air/aqueous solution interface evidenced a kinetic behavior compatible with the three steps model, induction regime, conformational change regime and relaxation regime coupled with a possible build-up of multilayers, proposed for various proteins [24]. On the other hand, the values at thermodynamic equilibrium of interfacial tensions are situated on a typical adsorption isotherm, thus evidencing the surface-active character of the investigated protein. The same character is manifested at liquid – liquid interfaces, studied on the model system, such as an aqueous NaCl solution with protein and stearic acid solution in benzene. Here the effect of both surface-active substances is conjugated.

EXPERIMENTAL SECTION

Materials

The protein solution used is that of the major globular storage protein from aleurone cells of barley (*Hordeum vulgare* L.) extracted and purified as shown elsewhere [31]. The protein was dissolved in ultra pure water and diluted to the working concentrations. The pH of the major aleurone protein solution was about 5.6. The protein is related to 7S globulins present in other cereals and to the vicilin-type 7S globulins of legumes and cottonseed. It contains 4 subunits of about 20, 25, 40 and 50 kDa molecular weights [31]. The N-terminal sequence of 16 amino acids in the protein [14] is given as follows: ^{1}*X* ^{2}*Glu* ^{3}*Gln* ^{4}*Gly* ^{5}*Asp* ^{6}*Ser* ^{7}***Arg*** ^{8}***Arg*** ^{9}*Pro* ^{10}*Tyr* ^{11}*Val* ^{12}*Phe* ^{13}*Gly* ^{14}*Pro* ^{15}***Arg*** ^{16}(*Ser* or *His*) ^{17}*Phe*, where *X* stands for the first amino acid of the N-terminal of aleurone protein which was not identified. The secondary structure of this protein was recently investigated by advanced spectroscopy [37, 38]. Deionized water of ultra high purity was used in all experiments and it was obtained from an Elgastat water purification system. Benzene, stearic acid and NaCl were of high purity, purchased from Merck and used without further purification.

Methods

The adsorption of the protein at the air/water interface was investigated by measurements of the dynamic interfacial tension of aqueous solutions with variable protein concentrations, in the range from 0.5 to 5 mg/L, by means of the ring method (Le Compte du Nouy) and the plate method (Wilhelmy method), described elsewhere [16-18, 39-41]. In order to maintain constant

the ionic strength of the aqueous phase and full solubility of globular protein, this aqueous phase was a 0.5 M NaCl solution [31]. The temperature was maintained constant at about 20 ^{o}C.

The influence of protein adsorption on interfacial tension at a water/ oil interface was studied using the following phases:

- benzene solutions of stearic acid (SA), having different SA concentrations in the range from 0.4 to 0.025 M
- an aqueous 0.5 M NaCl solution without protein and separately with a constant protein content of 2.9 mg/L.

The measurements of interfacial tension were executed by the ring method (Le Compte du Nouy) and the plate method (Wilhelmy) at two different temperatures (20^{o}C and 36^{o}C) for the oil/water interfacial systems.

ACKNOWLEDGEMENTS

This research was financially supported by Romanian National Research Program PNCDI2, grant 41050.

REFERENCES

1. R. A. Ganzevles, R. Fokkink, T. van Vliet, M. A. Cohen Stuart, H. H. J. de Jongh, *Journal of Colloid and Interface Science,* **2008**, 317, 137.
2. E. V. Kudryashova, H. H. J. de Jongh, *Journal of Colloid and Interface Science,* **2008**, 318, 430.
3. J. Minones Conde, J. M. Rodriguez Patino, *Journal of Food Engineering*, **2007**, *78*, 1001.
4. Y. Zhang, Z. An, G. Cui, J. Li, *Colloids and Surfaces A*, *Physicochemical and Engineering Aspects,* **2003**, *223*, 11.
5. F. Monroy, F. Ortega, R. G. Rubio and M. G. Velarde, *Advances in Colloid and Interface Science,* **2007**, *134-135*, 175.
6. J. Gualbert, P. Shahgaldian, A. W. Coleman, *International Journal of Pharmaceutics*, **2003**, 257, 69-73.
7. T. Xu, R. Fu, L. Yan, *Journal of Colloid and Interface Science,* **2003**, 262, 342.
8. S. V. Dorozhkin, E. I. Dorozhkina, *Colloids and Surfaces A*, *Physicochemical and Engineering Aspects*, **2003**, *215*, 191.
9. A. N. Lazar, P. Shahgaldian, A. W. Coleman, *Journal of Supramolecular Chemistry*, **2001**, *1*, 193.
10. Y. Wang, X. Yin, M. Shi, W. Li, L. Zhang, J. Kong, *Talanta*, **2006**, 69, 1240.
11. W. Lu, Y. Zhang, Y. -Z. Tan, K.-L. Hu, X.-G. Jiang, S.-K. Fu, *Journal of Controlled Release*, **2005**, *107*, 428.

12. T. Maruyama, S. Katoh, M. Nakajima, H. Nabetani, T. P. Abbott, A. Shono, K. Satoh, *Journal of Membrane Science,* **2001**, *192*, 201.
13. S. Salgin, S. Takac, T. H. Ozdamar, *Journal of Membrane Science*, **2006**, 278, 251.
14. J. Tian, J. Liu, X. Tian, Z. Hu and X. Chen, *Journal of Molecular Structure*, **2004**, *691*, 197.
15. F. Wang, Z. Yang, Y. Zhou, S. Weng, L. Zhang, J. Wu, *Journal of Molecular Structure*, **2006**, *794*, 1.
16. P. Joos, A. Tomoaia-Cotisel, A. J. Sellers, M. Tomoaia-Cotisel, *Colloids and Surfaces B: Biointerfaces*, **2004**, *37*, 83.
17. M. Tomoaia-Cotisel, P. Joos, *Studia Universitatis Babes-Bolyai, Chemia*, **2004**, *49* (1), 35.
18. M. Tomoaia-Cotisel, D. A. Cadenhead, *Langmuir*, **1991**, *7*, 964.
19. M. Tomoaia-Cotisel, J. Zsakó, E. Chifu, D. A. Cadenhead, *Langmuir*, **1990**, *6* (1), 191.
20. M. Tomoaia-Cotisel, *Progress in Colloid and Polymer Scence.,* **1990**, *83*, 155.
21. E. Dickinson, B.S. Murray, G. Stainsby, *Journal of the Chemical Society, Faraday Transactions, 1*, **1988**, *84*, 871.
22. A. -P. Wei, J. N. Herron, J.D. Andrade, in “From Clone to Clinic” (D.J.A. Crommelin, H. Schellekens, Editors.), Kluwe Academic Publishers*,* Dordrecht, **1990**, pp. 305.
23. W. Van der Vegt, H.C. Van der Mei, H.J.Busscher, *Journal of Colloid and Interface Science,* **1993**, *156*, 129.
24 C. J. Beverung, C.J.Radke, H.W.Blanch, *Biophysical Chemistry*, **1999**, *81*, 59.
25. G. Lu, H.Chen, J.Li, *Colloids and Surfaces A*, *Physicochemical and Engineering Aspects*, **2003**, *215*, 25.
26. S. M. Mel’nikov, *Colloids and Surfaces B: Biointerfaces*, **2003**, *27*, 265.
27. C. J. Beverung, C. J. Radke, H. W. Blanch, *Biophysical Chemistry*, **1998**, *70*, 121.
28. K. B. Song, S. Damodaran, *Langmuir*, **1991**, *7*, 2737.
29. M. G. Ivanova, R. Verger, A. G. Bois, I. Panaiotov, *Colloids and Surfaces*, **1991**, *54*, 279.
30. V. Ducel, J. Richard, Y. Popineau, F. Boury, *Biomacromolecules*, **2004**, *5*, 2088.
31. T. Yupsanis, S. R. Burgess, P. J. Jackson. P. R. Shewry, *Journal of Experimental Botany.,* **1990**, *41*, 385.
32. M. Tomoaia-Cotisel, in “Convergence of Micro-Nano-Biotechnologies, Series in Micro and Nanoengineering” Vol. 9 (Editors: M. Zaharescu, E. Burzo, L. Dumitru, I. Kleps, D. Dascalu), Romanian Academy Press, Bucharest, **2006**, pp. 147-161.
33. M. Tomoaia-Cotisel, A. Tomoaia-Cotisel, T. Yupsanis, Gh. Tomoaia, I. Balea, A. Mocanu, Cs. Racz, *Revue Roumaine de Chimie,* **2006**, *51*, 1181.
34. O. Horovitz, A. Mocanu, Gh. Tomoaia, L. Olenic, Gg. Mihăilescu, O. Boroştean, A. Popoviciu, C. Crăciun, T. Yupsanis, M.Tomoaia-Cotişel, In: "Convergence of micro-nano-biotechnologies, Series in Micro and Nanoengineering", Vol.9, Ed. Academiei, Bucureşti, **2006**, pp. 133-147.

35. O. Horovitz, Gh. Tomoaia, A. Mocanu, T. Yupsanis, Tomoaia-Cotisel, *Gold Bulletin*, **2007**, *40*, 213.
36. O. Horovitz, Gh. Tomoaia, A. Mocanu, T. Yupsanis, Tomoaia-Cotisel, *Gold Bulletin*, **2007**, *40*, 295.
37. I. Bratu, M. Tomoaia–Cotisel, G. Damian, A. Mocanu, *Journal of Optoelectronics and Advanced Materials,* **2007**, *9*, 672.
38. M. Tomoaia-Cotişel, A. Mocanu, N. Leopold, M. Vasilescu, V. Chiş, O. Cozar, *Journal of Optoelectronics and Advanced Materials,* **2007**, *9*, 637.
39. E. Chifu, M. Sălăjan, I. Demeter-Vodnár, M. Tomoaia-Cotişel, *Revue Roumaine de Chimie*, **1987**, *32* (7), 683.
40. M. Tomoaia-Cotisel, E. Chifu, in "*IUPAC Macro'83, Section IV: Structure and Properties"*, Bucharest, **1983**, pp. 636-639.
41. E. Chifu, M. Tomoaia-Cotisel, *Studia Universitatis Babes-Bolyai, Chemia*, **1987**, *26* (2), 3.
42. M. Tomoaia-Cotisel, D.A. Cadenhead, *Langmuir*, **1991**, *7*, 964.
43. G. Colacicco, *Journal of Colloid and Interface Science,* **1969**, *29*, 345.