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SHORT COMMUNICATION

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Monolayers of γ-globulin at the air–water interface

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Abstract Monolayer formation, of γ-globulin at the air–water interface has been investigated under varying subphase compositions. At pH 7.4, it is found that a stable monolayer is obtained only when the ionic strength is greater than 0.5 M. The magnitude of the collapse pressure increases with increasing ionic strength of the subphase. These data are analyzed in comparison to the literature data.

Key words γ-globulin · Monolayers · Molecular area

Introduction

Monolayer studies of γ-globulin films, at the air–water interface, are of great interest because knowledge can be gained about the formation of Langmuir–Blodgett films onto different solid supports. The latter can be useful for analyzing the possibilities of developing new biosensors by making oriented antibody molecular layers with well–defined and well-controlled characteristics [1, 2]. Moreover, by using the monolayer technique it is possible to get useful molecular information on the behaviour of this protein and additionally on the mechanisms involved in the adsorption characteristics on solid–liquid interfaces, for example, polystyrene–aqueous solution (latex) interfaces as a step in the development of an immunodiagnostic test [3, 4].

Furthermore, unlike most proteins, which may be spread quantitatively at air–water and oil–water interfaces to form very stable monolayers, the γ-globulin protein is found to spread at these interfaces only under some specific experimental conditions. In fact, very few systematic studies have been devoted to the investigation of the monolayers of this protein, and the data reported are not always consistent as regards the values of molecular area [5–8]. The magnitude of the area per molecule of an adsorbed protein at any interface is very important information, and must be determined quantitatively before any useful mechanisms for adsorbed proteins can be described [9].

The magnitude of the area per γ-globulin molecule as reported in the literature varies a great deal, from 0.1 to 1 m^2/mg. It is known [9] that most proteins at the interface spread to form a monolayer of about 1 m^2/mg. In the case of γ-globulin, only in one case has a value of 1 m^2/mg been reported [5].

The purpose of this study is to report the monolayer characteristics of γ-globulin at the air–water interface, with respect to the adsorption and the unfolding characteristics, as a function of subphase ionic strength.

Experimental

Materials

The protein used, γ-globulin from human blood, was supplied by Sigma as crystalline material. All chemical used were of reagent grade.

Experimental equipment and method

The monolayers of the γ-globulin protein, at the air–aqueous solution interface, were studied by the analysis of the surface pressure (π) versus the specific surface area (A_{sp}, m^2/mg) isotherms, which were obtained by measuring the change in surface pressure (at constant area, 149 cm^2), by the Wilhelmy-plate method, on the addition of an increasing amount of protein solution, as described elsewhere [9–11]. The surface pressure was measured using a platinum plate attached to a Sartorius model 4120 electrobalance. The range of sensitivity of the balance was ± 0.0025 dyn/cm.

Each experiment was performed as twice or more until isotherms with high reproducibility were obtained. In general, for all the systems studied, a high reproducibility of the results was obtained.

The protein spreading solution was a phosphate buffer, pH 7.4, ionic strength $I = 0.1$ M (physiological conditions). A Hamilton syringe, with a short needle, coupled to an "adding system", was used to enable the addition of 0.5 μl of the solution, in order to spread the protein solution on the surface.

The subphase in all the experiments was a phosphate buffer (pH 7.4). The ionic strength was varied (0.1, 0.5, 2.0 and 4.0 M) by the addition of NaCl. All the experiments were carried out at 25 °C.

Results and discussion

π versus A_{sp} isotherms for γ-globulin monolayers were measured. The parameters investigated were the effect of the subphase ionic strength (0.1, 0.2, 2 and 4 M) and the effect of the protein spreading solution concentration (0.5, 1 and 2 g/l).

The π versus A_{sp} experimental isotherms as measured at different ionic strengths of the subphase (0.1–4 M) for two protein spreading solution concentrations, 1 and 2 g/l, in a phosphate buffer (pH 7.4) are shown in Fig. 1. It is interesting to note that independent of the protein solution concentration, when the ionic strength of the subphase is 0.5 M or lower it is not possible to form stable monolayers. This means that most of the protein is lost into the subphase. However, when the ionic strength is 2 or 4 M the molecular area values are between 1 and 2 m^2/mg, in agreement with usual data for protein monolayers [9].

The π versus A_{sp} isotherms obtained for different protein solution concentrations at two ionic strengths (2 and 4 M) are shown in Fig. 2. It can be observed that, in both cases, the concentration of the protein solution (0.5–2 g/l) does not have a significant effect on the films. This result confirms the fact that when the ionic strength of the subphase is relatively high (above 2 M) it is possible to form stable monolayers of γ-globulin at the interface. Moreover, all the isotherms appear with similar limiting molecular area values, A_0, around 1 m^2/mg; this agrees with an early reported value [5].

On the other hand, the γ-globulin monolayer data reported in the literature show a large variation in the magnitude of the area per molecule (Table 1).

Considering γ-globulin as a protein with a molecular weight of 150000 D and 1320 residues/molecule [12] one gets a value of $A_0 = 18$–19 $Å^2$/residue (1 m^2/mg). This

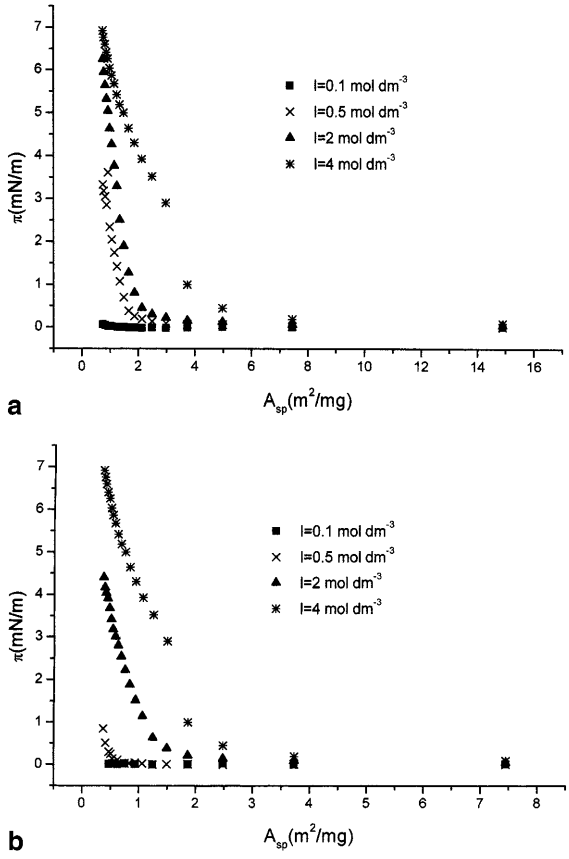

Fig. 1a, b Surface pressure (π) versus specific surface area (A_{sp}) isotherms for monolayers of γ-globulin spread on subphases with varying ionic strength. Protein spreading solution concentrations: **a** 1 g/l; **b** 2 g/l

value is comparable to the approximately 15 $Å^2$/residue value from X-ray diffraction data for various proteins [9, 11]. This result could indicate that the γ-globulin protein unfolds almost completely when it is spread on aqueous salt solutions. This leads to the conclusion that at the low surface pressure range and for protein spreading solution in physiological conditions, it is necessary to increase the ionic strength of the subphase to more than 0.5 M in order to form stable monolayers of γ-globulin. Furthermore, it is observed that the magnitude of the collapse pressure increases when the ionic strength increases from 2 to 4 M increasing the stability of the monolayers at the interface. These data will be the subject of future reports.

The magnitudes of the γ-globulin molecule dimensions reported by different authors are 66.5 [6], 45–125 [13] and 32–130 nm^2/molecule [14]. Considering that an area of 1 m^2/mg corresponds to a molecular area of 249 nm^2/molecule, it is possible to conclude from our results that, in fact, this protein is unfolded at these high ionic strengths, probably losing part of its secondary structure, and so that the protein spreads at the interface

Table 1 Limiting molecular area values, A_0, from different studies on monolayers of γ-globulin at the air–aqueous solution interface. The sample (*S*), spreading solution (*SS*) and the subphase (*Su*) are indicated. The immuno γ-globulin is represent by *IgG*

Ref.	Experimental conditions	A_0
[5]	s: bovine γ-globulin	
	ss: 1 g/l in 0.5 M sodium acetate, with 10% isopropyl alcohol	
	su: 0.1 N HCl (pH 1.4)	0.95 m^2/mg[a]
	su: 0.1 N NaCl (pH 8.9)	0.95 m^2/mg[a]
	su: 0.1 N NaOH (pH 12.5)	1.25 m^2/mg[a]
[6]	s: normal rabbit lgG	70 nm^2/molecule
	s: antilipid A rabbit lgG	14 nm^2/molecule
	ss: aqueous solution with 0.1% amyl alcohol	
	su: 0.15 M NaCl, pH 6.4	
[7]	s: polyclonal human lgG	40–80 nm^2/molecule[a]
	ss: 0.30 ml of a 0.6 g/l solution in aqueous isopropyl alcohol (60%v/v)	
	su: 0.1 M phosphate buffer solution (pH 6.6)	
[8]	s: polyclonal goat antirabbit lgG F(ab′)$_2$	35–45 nm^2/molecule[a]
	s: monoclonal antiprolactin	40–55 nm^2/molecule[a]
	ss: 0.3 ml of a 0.5 g/l pure water solution	
	su: phosphate subphases buffered at their isoelectric point	

[a]Estimated values from surface pressure versus specific surface area isothems

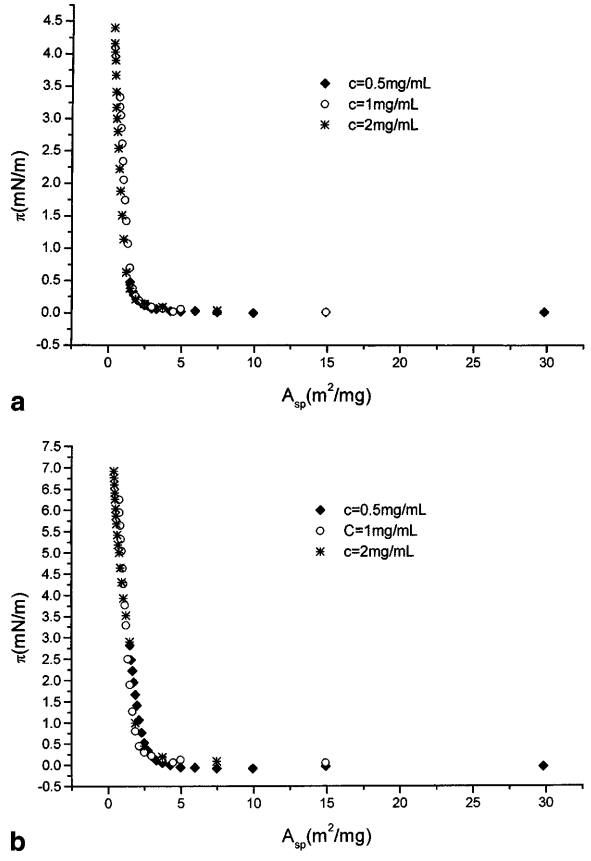

Fig. 2a, b Surface pressure (π) versus specific surface area (A_{sp}) isotherms for monolayers of γ-globulin spread on subphases with varying protein spreading solution concentrations. Ionic strength of the subphase: **a** $I = 2$ M; **b** $I = 4$ M

to form stable monolayers. In contrast, at low ionic strength a loss of the protein into the subphase cannot be excluded. This could explain the low limiting molecular area values observed in Fig. 1 and in Table 1. We suggest that this behaviour of the ionic strength is due to the protein structure. γ-globulin is relatively big and hydrophobic compared with other proteins. Moreover our sample is polyclonal. At a definite pH, different proportions of molecules charged with opposite sign should be in solution giving rise to precipitation. These facts could explain the difficulty in obtaining aqueous solutions of this protein at low ionic strength. However, the γ-globulin structure contains two very well defined parts, a polar and hydrophilic part, the F(ab′)$_2$ fragment, and an apolar and hydrophobic part, the F_c fragment. Hydration forces are widely recognized as strong stabilizers for hydrophilic surfaces [15–17]. An increase in bulk salt concentration should lead to adsorption of hydrated ions at the polar part of the protein, thus setting a barrier for intermolecular aggregation [18–20]. In this way the formation of a stable monolayer of protein would be favoured, taking into account the amphiphilic character of the protein.

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