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ORIGINAL CONTRIBUTION

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Interfacial behaviour of wheat puroindolines: monolayers of puroindolines at the air–water interface

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Abstract Puroindolines are among the major basic and cysteine-rich lipid binding proteins of wheat seeds. The interfacial properties of puroindoline-a (PIN-a) and puroindoline-b (PIN-b) are important both from a biological and a technological point of view. In the work reported here, the interfacial characteristics of spread monolayers of wheat puroindolines at the air–water interface were studied at varying subphase compositions using a Langmuir–Blodgett film balance. The compression isotherms (π–A_{Sp}) were recorded at constant barrier speed (3.3 cm/min). It was observed that both PIN-a and PIN-b form stable monolayers at the air–water interface. The stability of the monolayers was found to be dependent on the subphase composition as well as on the concentration of protein in the spreading solution. When the ionic strength of the subphase is below 0.50, the compression isotherms of both PIN-a and PIN-b remains unaffected with the change in the ionic strength of the subphase; however, when the ionic strength is above 0.50, the compression isotherms of both PIN-a and PIN-b undergo significant change with an increase in the ionic strength of the subphase. A gradual increase in the values of the collapse pressure (π_C) and the limiting area (A_0) was observed due to an increase in the ionic strength of the subphase from 0.5 to 4.0, which may be correlated with the salt-induced conformational changes of the protein molecule. The presence of NaCl and KCl (ionic strength 1.0) in the subphase has a comparable effect on the compression isotherms of both PIN-a and PIN-b; however, the presence of $CaCl_2$ (ionic strength 1.0) in the subphase leads to an increase in the values of π_C and A_0. A change in the pH of the subphase from 3.0 to 7.2 was to have a significant effect on the values of π_C and A_0, which may be due to the pH-induced alteration of the protein conformation.

Key words Wheat Proteins · Puroindolines · Monolayers · Air–water interface · Surface rheology

Introduction

Puroindolines are among the major basic cysteine-rich lipid binding proteins of wheat seeds which were first isolated from wheat endosperm using Triton X114 phase partitioning [1, 2]. Puroindolines isolated from wheat seeds consist of two major isoforms, puroindoline-a (PIN-a) and puroindoline-b (PIN-b). Amino acid sequencing of PIN-a revealed that there are 115 amino acid residues and five disulphide bridges in the polypeptide chains with a unique tryptophan-rich domain (WRWWKWWK). The primary structure of PIN-b was determined from the corresponding cDNA sequencing [3]. Both PIN-a and PIN-b exhibit 60% homology in their amino acid sequence in the polypeptide chain but the tryptophan-rich domain in the polypeptide chains of PIN-b is truncated (WPTKWWK). The structure–function relationship of puroindolines along with other cereal lipid binding proteins has been reviewed by Douliez et al. [4].

Like thionins [5, 6], puroindolines isolated from wheat have interesting antifungal properties [7] owing to their unique lipid-binding properties through tryptophan-rich domains [8–12]. They also have interesting surface-active and foaming properties [8, 11]. Both PIN-a and PIN-b form stable foams [8, 11]. The lipid-binding, surface-active and foaming properties of puroindolines make them protein molecules of interest from both biological and technological standpoints. The interfacial properties of puroindolines along with other wheat proteins are of utmost important in the cereal-based industry, for example, in bread, cakes and other bakery products [13, 14]. The importance of interfacial properties of wheat proteins has recently been reviewed by Örnebro et al. [15]. The rheology of bread dough and the size of the air cells depend on the concentration of puroindolines in the wheat flour [14]. For a fundamental understanding of the effect of puroindolines on the rheology of bread dough, it is essential to obtain information on their packing at the interface. Such information can be obtained from pressure–area (π–A_{Sp}) isotherms of the spread monolayers of these two proteins at the air–water interface [16–19].

Thus, the study of interfacial properties of puroindolines is of fundamental and technological importance and the matter needs systematic investigations. The interfacial properties of puroindolines were studied earlier by us [7, 8, 10, 11] and others [20]; however, studies on monolayer properties of puroindolines are rare. In our present work, we have undertaken to study the monolayer properties of puroindolines at the air–water interface at varying subphase compositions using a Langmuir–Blodgett trough. From the π–A_{Sp} isotherms, the different parameters, the collapse pressure (π_C), the limiting area (A_0) and the surface dilatational elasticity modulus (E), which determine the stability of protein monolayer were calculated. The interfacial properties of PIN-a and PIN-b were also compared with each other.

Materials and methods

The PIN-a and PIN-b used in this work were isolated from wheat seeds (*Triticum aestivum*, cv Etoile de Choisy). The ultrapure deionized water used in this work was purified using a Millipore filtration device (Milli Q Systems). All other chemicals used in this work were of analytical grade. The surface tensions of aqueous buffered solutions were checked by surface tension measurement using a tensiometer and buffer solutions having accepted surface tension in the literature (72–73 mN/m at ambient temperature) were used.

Puroindolines were purified according to a modification of an earlier procedure [1, 10], where five kilogram of wheat seeds (*T. aestivum*, cv Etoile de Choisy) were ground and extracted with 20 l 100 mM tris(hydroxymethxl)aminomethane(Tris), HCl buffer of pH 7.8 containing 100 mM KCl, 5 mM ethylenediaminetetraacetate and 4% Triton X114 [1]. The mixture was kept overnight at 4 °C and centrifuged at 5000*g* for 30 min. The supernatant was heated to 30 °C for phase separation. The heavy detergent-rich phase was isolated from the detergent-poor phase by centrifugation at 5000*g* for 15 min at 30 °C and was diluted with 40 l 50 mM Tris, HCl buffer of pH 6.8. This diluted phase was then loaded on a column (50 × 220 mm) packed with a cation exchanger (Sepharose Sp big beads, Pharmacia Bio–Tech). The proteins were eluted by applying a 0–1 M NaCl gradient in 50 mM Tris, HCl buffer of pH 6.8 and detected at 280 nm using an absorbance detector. The fractions containing puroindolines were dialysed (Spectra/Por membrane with a cut off molecular weight of 6000–8000 Da) in deionized water at 4 °C with periodical change of water. The solution of purified total puroindolines (PIN-a and PIN-b) was freeze-dried and stored at room temperature. PIN-a and PIN-b were isolated from total protein by fast protein liquid chromatography as described previously by Blochet et al. [1] and the purity of PIN-a and PIN-b was checked by both sodium dodecyl sulfate–polyacrylamide gel electrophoresis and reverse-phase high-pressure liquid chromatography. The protein content of the isolated fractions of PIN-a and PIN-b were measured by Kjeldahl analysis and both PIN-a and PIN-b were found to be 98.9% pure. The accurate molecular masses of PIN-a and PIN-b was determined using a triple quadruple mass spectrometer (APII, Sciex, Toronto, Canada).

The monolayers of puroindolines at the air–water interface were studied from the isotherms of surface pressure (π) versus the specific surface area (A_{Sp}) using a computer-controlled and user-programmable Langmuir–Blodgett Teflon-coated trough (KSV5000, equipped with a single movable barrier of total surface area 0.0724 m^2). The surface pressure was measured following a Wilhelmy-plate method using a roughened platinum plate connected to a microelectronic feedback system for surface pressure measurement. Before starting the experiment, the trough was cleaned successively with deionized water, chloroform and finally with ultrapure deionized (Millipore) water. The trough was then filled up by the subphase (pH solution) and the air–water interface was freed from impurities by repeated aspiration and checking the π–A_{Sp} isotherms each time. When the π–A_{Sp} isotherms exhibited a good baseline during compression, it was confirmed that the air–water interface was cleaned properly. After cleaning, the protein was spread at the air–water interface by careful addition of a small volume (20 μl) of a concentrated solution (e.g. 2 g/l) prepared by dissolving an accurate amount of protein in buffer solution at the air–water interface using a microsyringe [21, 22]. The system was kept undisturbed for 45 min for complete spreading, adsorption and rearrangement of the proteins at the air–water interface. The spread protein layer was then compressed by moving the barrier at a rate of 3.3 cm/min, which was the highest speed where the compression isotherms were found to be highly reproducible [22–24]. Each experiment was repeated at least three times by making up the solution and preparing the trough from the beginning until a high reproducibility of the π–A isotherm was obtained. The reproducibility of the surface pressure value was found to be ±0.4 mN/m. The temperature of the system during the experimental work was kept fixed at 25 ± 0.5 °C using a circulating water bath. All the glass apparatus used in this experiment were washed successively with chromic acid, ultrapure deionized water and ethanol and finally with ultrapure deionized water.

Results

The compression isotherms (π–A_{Sp}) of puroindolines (PIN-a and PIN-b) measured at four different concentration of protein, C_{P} (1.0, 1.5, 2.0 and 2.5 g/l), in the spreading solution are presented in Fig. 1. One notes that both PIN-a and PIN-b spread very well at the air–water interface and they formed stable monolayers even at 1.0 g/l concentration of protein in the spreading solution. The shape of the compression isotherms of both PIN-a and PIN-b depended on the concentration of protein in the spreading solution. At 1.0 g/l concentration of protein, the isotherms were smooth without showing any inflection point due to a liquid condensed phase [25]; however, when the value of C_{P} increased from 1.5 g/l to 2.5 g/l, there appeared an inflection point in the compression isotherms, which was prominent at 2.5 g/l. Thus, when the concentration of protein in the spreading solution was relatively high, the compression isotherms of puroindolines exhibited an inflection point which may be correlated with the formation of a liquid condensed phase [25] involving protein–protein interaction at the air–water interface. From Fig. 1, it is noteworthy that with increasing values of C_{P}, the values of the collapse pressure (π_{C}) increased gradually but the limiting area (A_0) remained unchanged. A_0 represents the minimum area of the expanded monolayer of protein film at the air–water interface which can be calculated by graphical extrapolation to $\pi = 0$ on the area axis. The values of π_{C} and A_0 determined from the π–A_{Sp} isotherms of PIN-a and PIN-b are presented in Table 1 for comparison.

The π–A_{Sp} compression isotherms of PIN-a and PIN-b as measured at different ionic strength (0.10–4.0, maintained by NaCl) for a protein spreading solution of concentration 2.0 g/l are presented in Fig. 2. From Fig. 2,

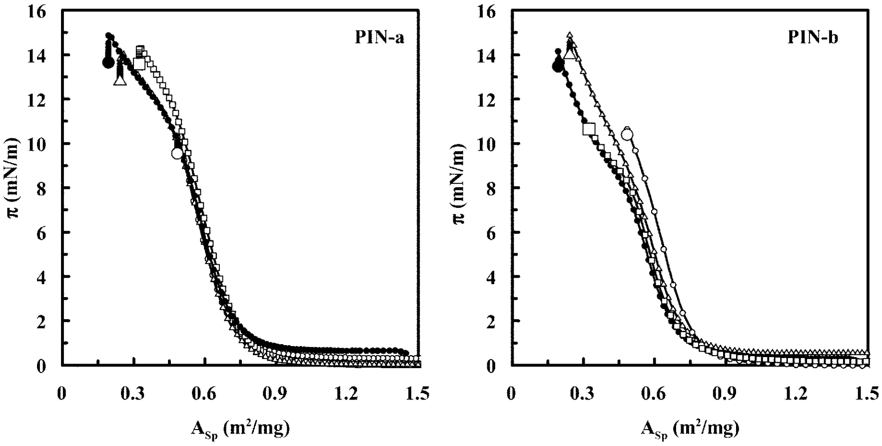


Fig. 1 Compression isotherms (π–A_{Sp}) of wheat puroindoline-a (*PIN-a*) and puroindoline-b (*PIN-b*) at the air–water interface as measured for different concentration of protein in the spreading solution at pH 7.2 (10 mM phosphate), barrier speed 3.3 cm/min and 25 °C (*left* PIN-a, *right* PIN-b). 1.0 g/l (○); 1.5 g/l (□); 2.0 g/l (△); 2.5 g/l (●)

Table 1 Collapse pressure (π_{C}), limiting area (A_0) and elasticity modulus (E) for puroindolines at the air–water interface at different physicochemical conditions

C_{P} (g/l)	Subphase	Puroindoline-a			Puroindoline-b		
		π_{C} (mN/m)	A_0 (m^2/mg)	E (mN/m)	π_{C} (mN/m)	A_0 (m^2/mg)	E (mN/m)
1.0	PH 7.2, 10 mM phosphate	12.4	0.747	24.5	10.7	0.781	24.3
1.5	PH 7.2, 10 mM phosphate	14.4	0.766	24.8	10.7	0.725	21.0
2.0	PH 7.2, 10 mM phosphate	14.3	0.742	24.8	14.9	0.750	21.2
2.5	PH 7.2, 10 mM phosphate	15.1	0.767	24.9	14.2	0.723	19.7
1.0	PH 7.2, μ = 0.10	10.1	0.756	23.2	11.0	0.780	25.2
1.0	pH 7.2, μ = 0.50	10.8	0.816	21.3	12.1	0.849	25.2
1.0	pH 7.2, μ = 1.0	14.8	0.853	21.1	14.5	0.885	27.9
1.0	pH 7.2, μ = 2.0	15.4	0.910	20.8	16.3	0.981	29.2
1.0	pH 7.2, μ = 4.0	19.5	1.14	20.9	19.8	1.06	29.3
2.0	pH 7.2, μ = 0.10	12.6	0.730	23.9	11.1	0.756	19.7
2.0	pH 7.2, μ = 0.50	13.2	0.761	23.8	11.6	0.766	22.9
2.0	pH 7.2, μ = 1.0	18.1	0.936	29.8	13.4	0.772	26.5
2.0	pH 7.2, μ = 2.0	16.5	0.791	23.4	14.8	0.791	28.3
2.0	pH 7.2, μ = 4.0	22.3	0.936	23.3	20.9	0.875	32.1
2.0	pH 3.0, phosphate–citric acid	14.2	0.698	27.3	13.2	0.603	21.4
2.0	1.0 M KCL, μ = 1.0	17.9	0.957	29.8	14.2	0.691	25.5
2.0	0.5 M $CaCl_2$, μ = 1.0	22.2	1.20	27.1	20.2	1.05	20.1

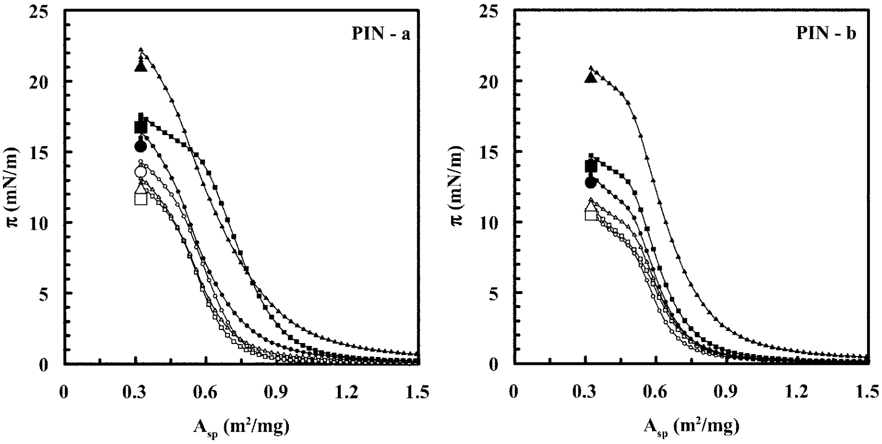


Fig. 2 Compression isotherms (π–A_{Sp}) of wheat PIN-a and PIN-b at the air–water interface as measured for different ionic strength (maintained by NaCl) of the subphase for protein spreading solution of concentration 2.0 g/l at pH 7.2, barrier speed 3.3 cm/min and 25 °C (*left* PIN-a, *right* PIN-b). $\mu = 0.01$ (○); $\mu = 0.10$ (□); $\mu = 0.50$ (△); $\mu = 1.0$ (●) and $\mu = 2.0$ (■); $\mu = 4.0$ (▲)

one notes that when the ionic strength of the subphase was below 0.50, there was no significant variation in the compression isotherms of PIN-a and PIN-b; however, when the ionic strength of the subphase was relatively high (above 0.50), the compression isotherms for both PIN-a and PIN-b exhibited significant changes in the values of π_C and A_0. As the ionic strength of the subphase increased from 0.50 to 4.0, both π_C and A_0 underwent a gradual shift towards higher values; this could be correlated with the salt-induced unfolding of protein molecules at high concentration [26–28]. Thus, the ionic strength of the subphase had an important bearing on the stability of monolayer films of PIN-a and PIN-b formed at the air–water interface. The values of π_C and A_0 associated with the compression isotherms of PIN-a and PIN-b were determined and are included in Table 1. The same trend in the π–A_{sp} isotherms was observed when the measurement was made at a protein spreading solution of concentration 1.0 g/l (not shown, see Table 1 for the values of π_C and A_0).

Compression isotherms of PIN-a and PIN-b at two different pH (3.0 and 7.2) of the subphase for a spreading solution of concentration 2.0 g/l of protein are shown in Fig. 3. On shifting the subphase pH from 7.2 to 3.0, the compression isotherms of both PIN-a and PIN-b underwent a contraction in the limiting area but the values of π_C remained almost unchanged. The values of π_C and A_0 were calculated and are included in Table 1.

The compression isotherms of PIN-a and PIN-b were recorded in the presence of different salts (NaCl, KCl and $CaCl_2$) in the subphase (ionic strength maintained at 1.0) for a protein spreading solution of concentration 2.0 g/l. The compression isotherms are presented in Fig. 4 for comparison. It is interesting to note that the presence of NaCl and KCl (ionic strength 1.0) in the subphase has a comparable effect on the compression isotherms of PIN-a and PIN-b; however, the presence of $CaCl_2$ (ionic strength 1.0) in the subphase had a remarkable effect on the shape of the compression isotherms of both PIN-a and PIN-b. The transition region between the liquid-expanded and the liquid condensed phase became more prominent. There was an increase in the values of π_C and A_0 in presence of $CaCl_2$ compared to the values in presence of NaCl and KCl. The values of π_C and A_0 are included in Table 1.

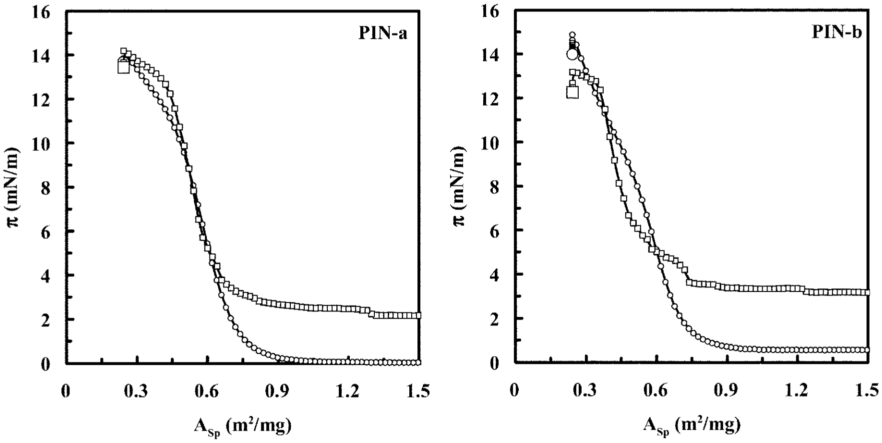


Fig. 3 Compression isotherms (π–A_{Sp}) of wheat PIN-a and PIN-b at the air–water interface as measured at two different pH of the subphase for a protein concentration of 2.0 g/l in the spreading solution at a barrier speed of 3.3 cm/min and 25 °C (*left* PIN-a, *right* PIN-b). pH 7.2 (○); pH 3.0 (□)

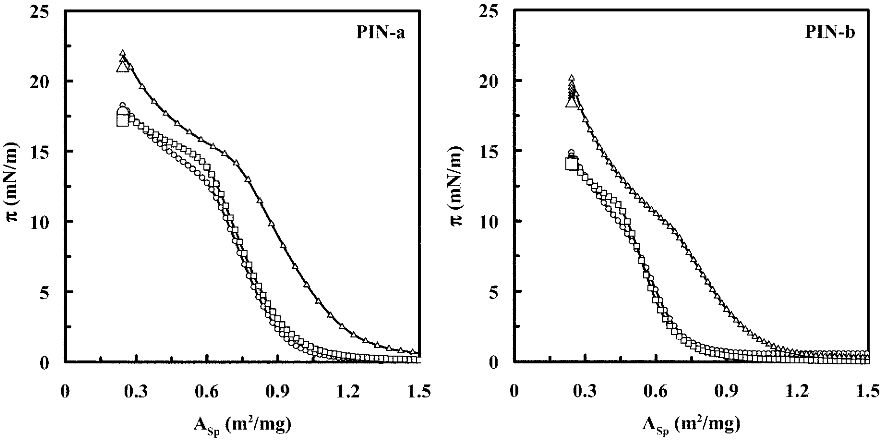


Fig. 4 Compression isotherms (π–A_{Sp}) of wheat PIN-a and PIN-b at the air–water interface as measured in the presence of different salts (NaCl, KCl and $CaCl_2$) in the subphase for a protein spreading solution of concentration 2.0 g/l at a barrier speed of 3.3 cm/min and 25 °C (*left* PIN-a, *right* PIN-b). NaCl (○); KCl (□); $CaCl_2$ (△)

The variation of π_C as a function of subphase composition is summarized in Fig. 5.

The surface dilatational modulus (E) related to the change in surface pressure (π) per unit change in the interfacial area is expressed as $E = -A(\mathrm{d}\pi/\mathrm{d}A)$, where A is the area of the protein film and π is the surface pressure [29]. Thus, E can be calculated from the π–A_{Sp} isotherm of a spread monolayer of puroindoline. The values of E for PIN-a and PIN-b at different physicochemical conditions are included in Table 1 and the variation of E with the composition of the subphase is presented in Fig. 6. From Fig. 6, one notes that the values of E for PIN-a are independent of the ionic strength of the subphase, whereas for PIN-b an increase in the ionic strength of the subphase leads to an increase in the value of E. The presence of Na^+ and K^+ has an identical effect on the values of E for both PIN-a and PIN-b; however, the presence of Ca^{2+} in the bulk medium has the opposite effect on the values of E for monolayers of PIN-a and PIN-b at the air–water interface. The effect of pH on the values of E are not significant.

Discussion

From primary structural and amino acid sequence analysis, it has been found that PIN-a and PIN-b contain ten cysteine residues with 60% homology in their primary structure [3]. Secondary structure analysis using Fourier transform (FT) IR spectroscopy [30] reveals that both PIN-a and PIN-b have almost the same secondary structure. The polypeptide chains in the tertiary structure are held together by five disulphide bonds involving ten cysteine residues. Both proteins contain a tryptophan-rich domain, which is partially truncated in PIN-b. So both proteins have similar structural features, though their polypeptide backbones are 60% homologous in terms of the primary amino acid sequence. Husband et al. [11] have shown that both PIN-a and PIN-b have comparable foaming properties with the same surface elasticity modulus, 18.2 mN/m.

The efficiency of a protein molecule to spread and form a stable monolayer at the air–water interface can be checked using protein spreading solution of different concentration [21]. The π–A_{Sp} compression isotherms

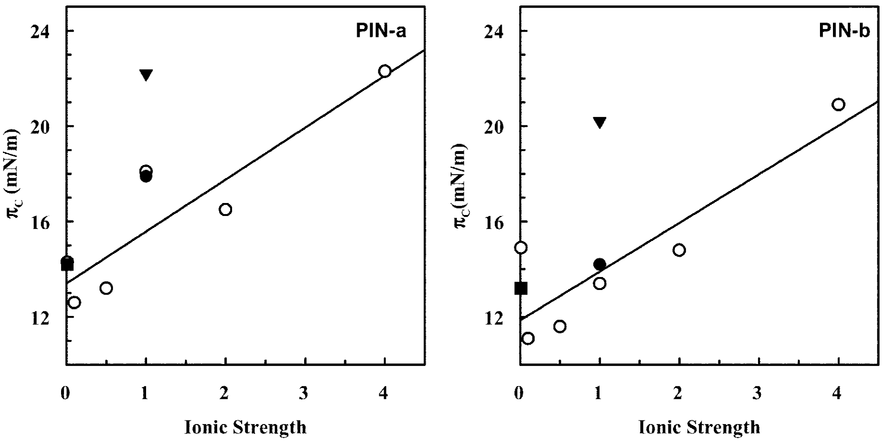
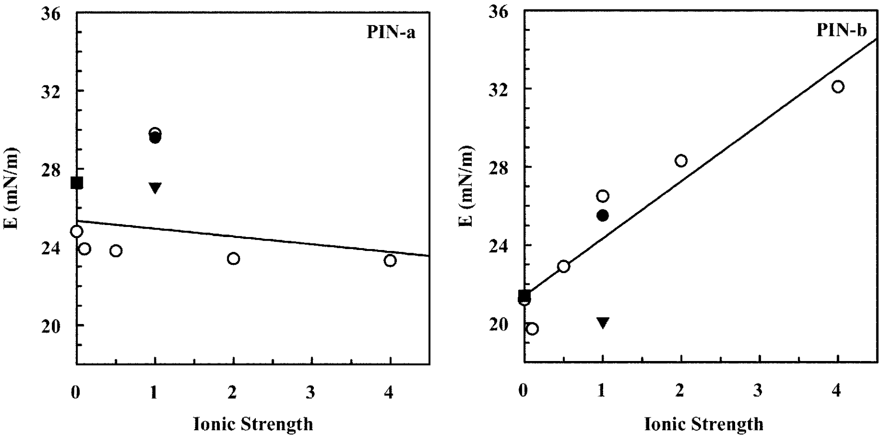


Fig. 5 Collapse pressure, π_C, of PIN-a and PIN-b as a function of subphase composition for a protein spreading solution of concentration of 2 g/l at 25 °C with a barrier speed 3.3 cm/min (*left* PIN-a, *right* PIN-b). Ionic strength maintained by NaCl (○) by KCl of ionic strength 1.0 (●), by $CaCl_2$ of ionic strength 1.0 (▼) and pH 3.0 (■)

Fig. 6 Elasticity modulus of PIN-a and PIN-b as a function of subphase composition for a protein spreading solution of concentration 2 g/l at 25 °C with a barrier speed of 3.3 cm/min (*left* PIN-a, *right* PIN-b). Ionic strength maintained by NaCl (○), by KCl of ionic strength 1.0 (●), by $CaCl_2$ of ionic strength 1.0 (▼) and pH 3.0 (■)



measured at four different concentration of PIN-a and PIN-b are highly reproducible, which indicates that both PIN-a and PIN-b have high efficiency for spreading at the air–water interface and both of them form stable monolayers at the air–water interface. It is interesting to note that the limiting areas for PIN-a and PIN-b are around 0.75 m^2/mg, which is close to the molecular area values for other proteins [31, 32]. Considering the molecular weights of PIN-a and PIN-b to be 12,835 and 13,080 with 115 and 116 amino acid residues, respectively, in the polypeptide chain, the monolayer area of PIN-a and PIN-b is around 14 Å^2/residue, which is comparable to the approximately 15 Å^2/residue value obtained from X–ray diffraction data for many other proteins [32, 33]. Thus, like other globular proteins, PIN-a and PIN-b unfold almost completely at the air–water interface, though both protein molecules have a compact-folding structure stabilized by five disulphide bonds. One more interesting point to be noted (Fig. 1) is that with increasing concentration of protein in the spreading solution, an inflection point appears in the π–A_{Sp} isotherms of both PIN-a and PIN-b and this becomes prominent at a higher concentration of protein in the spreading solution. This may be due to the involvement of more protein–protein interactions with increasing concentration of protein at the air–water interface with the formation of a liquid condensed phase.

The ionic strength of the subphase has a significant effect on the π–A_{Sp} isotherms of the spread monolayers of both PIN-a and PIN-b. In the presence of salts, the protein molecules spread more easily at the interfaces and spread monolayers of proteins are best studied over a subphase containing a high salt concentration of an inorganic salt [26]. The ease of spreading and unfolding of protein molecules in the presence of a salt is generally explained either by salting-out phenomena [27] or by the diminution of intramolecular cohesion caused by reduced ionic attraction involving ionizable groups [28]. Both the values of π_C and A_0 increase significantly (considering a standard error of ±0.4 mN/m in the surface pressure measurement and ±0.003 m^2/mg in the measurement of the monolayer area) with the increase in ionic strength of the subphase (Fig. 2, Table 1). The variation of the A_0 values with the ionic strength of the medium for 1 g/l protein spreading solution (7 Å^2/residue for PIN-a and 6 Å^2/residue for PIN-b) is high compared to that for 2 g/l protein spreading solution (4 Å^2/residue for PIN-a and 2 Å^2/residue for PIN-b). An increase in the ionic strength of the medium leads to a decrease in the intramolecular electrostatic interaction within the protein molecule, which may induce an unfolded conformation in the protein structure. So, an increase in the ionic strength of the subphase leads to greater spreadability of puroindolines at the air–water interface.

The influence of ionic composition on the macromolecular stability is complex and the electrostatic free energy of a protein roughly scales with the net charge of the protein molecule [34]. At relevant physicochemical conditions, solvent ions provide a counterion atmosphere that serves to attenuate Coulombic interactions. In addition to this, solvent ions can function as discrete ligands, which can bind to different regions of the proteins. The two isoforms of wheat puroindolines, PIN-a and PIN-b, exhibit almost identical monolayer behaviour when the subphase contains either NaCl or KCl under comparable experimental conditions (Fig. 4). The values of π_C and A_0 for PIN-a and PIN-b remain almost the same in the presence of NaCl or KCl (ionic strength 1.0) in the subphase (Table 1). The presence of $CaCl_2$ (ionic strength 1.0) in the subphase has an interesting and significant effect on the shape of the π–A_{Sp} isotherms for both PIN-a and PIN-b (Fig. 4). The values of π_C and A_0 both for PIN-a and PIN-b in the presence of $CaCl_2$ are found to be higher than the values in the presence of NaCl or KCl in the subphase. The variation of π_C for different systems studied for a protein spreading solution of concentration 2.0 g/l is compared in Fig. 5. The transition region due to a liquid condensed phase is more pronounced and shifted to higher area values when the subphase contains $CaCl_2$. This may be attributed to the differential binding of charged ions by PIN-a and PIN-b which may lead to formation of a more open and unfolded structure of puroindolines. This is reflected in the increase in the values of A_0 for both PIN-a and PIN-b. In addition to this electrostatic effect, a high concentration of salts also has a nonspecific effect on the hydrophobic interaction; this may also induce a somewhat open structure owing to the breaking of the water structure surrounding the protein molecule. The extent of this perturbation of the water structure surrounding the protein molecule depends on the nature of the electrolytes and for cations the extent of this effect follows the order $Ca^{2+} > Mg^{2+} > NH_4^+ > Na^+ > K^+$ [35].

The π–A_{Sp} isotherms of both PIN-a and PIN-b are found to be displaced towards the surface pressure axis and the monolayer structures are more condensed at acidic pH (Fig. 3). The A_0 values for both PIN-a and PIN-b are lower at pH 3.0 than at pH 7.2 (Table 1). Thus the amino acid residues of both PIN-a and PIN-b are more close-packed at the air–water interface on an acidic subphase. Both PIN-a and PIN-b are basic proteins with calculated isoelectric points around 10.5 and 10.7, respectively. PIN-b is more charged than PIN-a. A decrease in the pH might lead to a reduction in the repulsive interaction between amino acid residues owing to a decrease in the effective charge of the protein molecules. From their study on the secondary structure and conformation of puroindolines using FTIR spectroscopy Le Bihan et al. [30] have shown that the conformations of PIN-a and PIN-b are sensitive to the pH of the bulk medium. From their studies, they observed that at pH 4.0 the α-helix content of both PIN-a and PIN-b is about 8% higher than that at pH 7.0. This increase in the α-helix content is compensated for by the equivalent decrease in the β sheets and the unordered structure content of the protein molecules. The decrease in A_0 values of PIN-a and PIN-b may be correlated with this pH-induced conformational change as found by Le Bihan et al. [30].

The surface rheological properties of adsorbed/spread layers of proteins are amongst their most important attributes [18, 19, 20, 21], which determine the stability of protein-based foam. The value of E is related to the stability of the monolayer at the air–water interface and a higher value of E indicates enhanced stability of the protein monolayers. The values of the elasticity modulus depend on the nature of the subphase. The variation of the elasticity modulus values for different subphase compositions is presented in Fig. 6. The spread monolayer of PIN-b has increasing values of E with an increase in the ionic strength of the subphase, whereas the value of E for PIN-a remains almost unchanged with an increase in the ionic strength of the subphase (Table 1, Fig. 6). Thus, the increase in the ionic strength of the subphase imparts more elasticity and stability to a monolayer of PIN-b than to that of PIN-a at protein spreading solutions of concentration 1.0 and 2.0 g/l. With the variation of the subphase pH from 7.2 to 3.0, the value of E increases from 24.8 to 27.3 mN/m for spread monolayer of PIN-a for a protein spreading solution of concentration 2.0 g/l; however, the value of E for PIN-b remains almost unchanged with the variation of pH of the subphase from 7.2 to 3.0 at a protein spreading solution of concentration 2.0 g/l. So, an acidic subphase may have a stabilizing effect on the monolayer of PIN-a compared to the monolayer of PIN-b. From Table 1, one also notes that the presence of Na^+ and K^+ ions in the subphase leads to an increase in the values of E, which means that both these ions have a stabilizing influence on the monolayers of PIN-a and PIN-b. The presence of Ca^{2+} in the subphase has a stabilizing effect on the monolayer of PIN-a at the air–water interface, whereas for PIN-b the value of E remains almost unaltered when the subphase contains calcium ion. This difference in the monolayer behaviour between PIN-a and PIN-b in the presence of different electrolytes may be correlated with their unequal affinities towards these cations.

Conclusions

From our experimental results it may be concluded that both PIN-a and PIN-b spread very well at the air–water interface. The monolayer characteristics of both PIN-a and PIN-b are dependent on the subphase composition. The values of π_C and A_0 for both PIN-a and PIN-b increase with the increase in the ionic strength of the subphase. The values of π_C is independent of the variation in the subphase pH, but the values of A_0 for both PIN-a and PIN-b decreases with the shift of the pH of the subphase from 7.2 to 3.0. The amino acid residues of PIN-a and PIN-b molecules at the air–water interface are more closely packed on an acidic subphase. The values of π_C and A_0 increase in the presence of Ca^{2+} in the subphase. The elasticity modulus of the PIN-a monolayer is independent of the ionic strength of the medium, but the elasticity modulus of the PIN-b monolayer increases with an increase in the ionic strength of the medium. So,the ionic strength of the subphase has a stabilizing influence on the monolayer of PIN-b. Our results may be correlated with the foaming properties of both PIN-a and PIN-b that were studied earlier by Dubreil et al. [7] and Husband et al. [11].

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