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Adsorption dynamics of L-glutamic acid copolymers at a heptane/water interface

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Abstract

Random copolymers of glutamic acid (glu–ala, glu–leu, glu–phe, glu–tyr) were employed to investigate the relationship between side chain structure and peptide charge on adsorption behavior at an oil/water boundary. Adsorption of a series of glutamate copolymers at a heptane/water interface was examined by the dynamic pendant-drop method to determine interfacial tension. Incorporation of leucine or phenylalanine into a glutamate copolymer results in greater tension reduction than incorporation of alanine or tyrosine. These effects are amplified at pH values near the isoelectric point of glutamate, where macroscopic adsorbed films of glu–leu and glu–phe exhibit gel-like properties in response to interfacial area compression. Differences in interfacial tension behavior of glu–tyr and glu–phe indicate the importance of the tyrosine *p*-hydroxyl group on adsorption and aggregation at the oil/water interface. © 1998 Elsevier Science B.V.

Keywords: Protein adsorption; Liquid/liquid interface; Interfacial tension; Polyamino acids

1. Introduction

The amphiphilicity of amino acid side chains renders most proteins surface active. Thus, exposure of an aqueous protein solution to a nonpolar phase invariably results in adsorption of protein molecules to the interface. While many studies focus on protein adsorption at aqueous/solid interfaces [1–5], less is known about adsorption at liquid/fluid interfaces, and the oil/water interface in particular. Protein adsorption at the oil/water boundary governs the formation and stabilization of food emulsions [6–10]. The interactions of proteins with oil interfaces are of interest in microemulsion and reverse micellar extraction techniques [11–13]. Structure and activity of proteins in micellar environments is also under investigation [14,15]. The relationship between protein structure and surface activity is not well understood and knowledge of the conformation of adsorbed proteins is essential to understand the long-term effects of an interface on enzymatic activity.

There are several distinct time regimes in interfacial tension resulting from the adsorption of proteins at air/water interfaces. These can be characterized as resulting from diffusion, conformational changes, and aggregation of proteins. The time constants range from seconds to hours and even days [16–19]. Globular proteins may not reach an equilibrium interfacial tension (e.g., [20]), and the adsorption process is believed to be irreversible [16,21–24].

The goal of this work is to probe the effects of

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amino acid side chain hydrophobicity, charge, and bulk conformation on adsorption dynamics at a heptane/water interface. Copolymers of glutamic acid serve as simple models of proteins and those used here contained only two monomers; glutamic acid and one of several test amino acids. This permits isolation of the adsorption mechanisms operative with proteins. There are several reports on the use of copolymer as models for protein adsorption. Spread monolayers of synthetic homopolymers and amphoteric copolymers at the air/water interface have been examined [25–28]. Films formed by proline and hydroxyproline copolymers have also been studied [29]. More recently, poly-L-lysine has been used to investigate temperature effects on the surface tension of very long chain (80 kDa) polypeptides [30], and to examine variations related to secondary structure of the polypeptide adsorbed at a water/dodecane interface [31].

In this work, dynamic interfacial tension is measured using pendant-drop tensiometry. The importance of the charge of the synthetic polymer on interfacial tension reduction is assessed by studies at different pH values. After exposure to the heptane phase, the interfacial area was reduced to observe any macroscopic viscoelastic adsorbed layers. This compression provides a qualitative indication of the presence or absence of cohesive interfacial layers.

2. Materials and methods

Random copolymers used in this work consisted of glutamic acid with alanine, leucine, phenylalanine, or tyrosine as comonomer. These comonomers encompass a representative range of chain length, aliphaticity, and hydrophobicity. The charged glutamate side chain improved copolymer aqueous solubility.

The four copolymers and a glutamic acid homopolymer were obtained from Sigma Chemical, St. Louis, and used without further purification. Mass average molecular weights reported by the supplier were based on size exclusion chromatography and low-angle laser light scattering (SEC-LALLS). Polymers comparable in molecular weight to proteins (30–60 kDa) were chosen for this study. Table 1 gives the physical data for the synthetic poly-amino acids, including hydrophobicities of the comonomer amino acids [32].

Copolymer concentrations of 15 μg/ml were used in this study. Copolymers were studied at pH 7.1 and pH 5.3 to observe charge effects on the tension dynamics and bulk copolymer structure. All poly-amino acids were found to be insoluble in water at pH below 5.0. Solutions were prepared in acid-washed glassware. Buffers employed were 100 mM sodium phosphate (Fisher certified ACS grade), and were prepared with Millepore deionized water. Heptane (99% spectrophotometric grade, Fisher) was purified by passing it through a roasted silica bead column, and contacted with pure buffer solution for 24 h to establish a water-saturated oil phase. The interfacial tension of the resulting purified heptane/water interface was in agreement with the literature value of 50.2 mN/m [33].

Dynamic interfacial tension was measured using the pendant drop technique ([34,35]). A schematic of

Table 1
Properties of glutamic acid copolymers

Polypeptide	Residue hydrophobicity (kcal/mol)[a]	Composition (ratio glu:X)	Molecular weight (approx)	Degree of polymerization
Poly-glutamate	−0.11	–	36,200	240
Poly-glutamate-alanine	0.25	58:42	30,000	240
Poly-glutamate-tyrosine	2.45	80:20	45,300	295
Poly-glutamate-phenylalanine	2.06	81:19	41,300	275
Poly-glutamate-leucine	2.58	85:15	60,200	420

[a] Expressed as $\Delta G_{transfer}$ (EtOH–H_2O). Average of four sets of data compiled by Nakai and Li-chan [32] for individual amino acid $\Delta G_{transfer}$ values. Normalized to glycine (side chain-H) value of 0.0 kcal/mol.

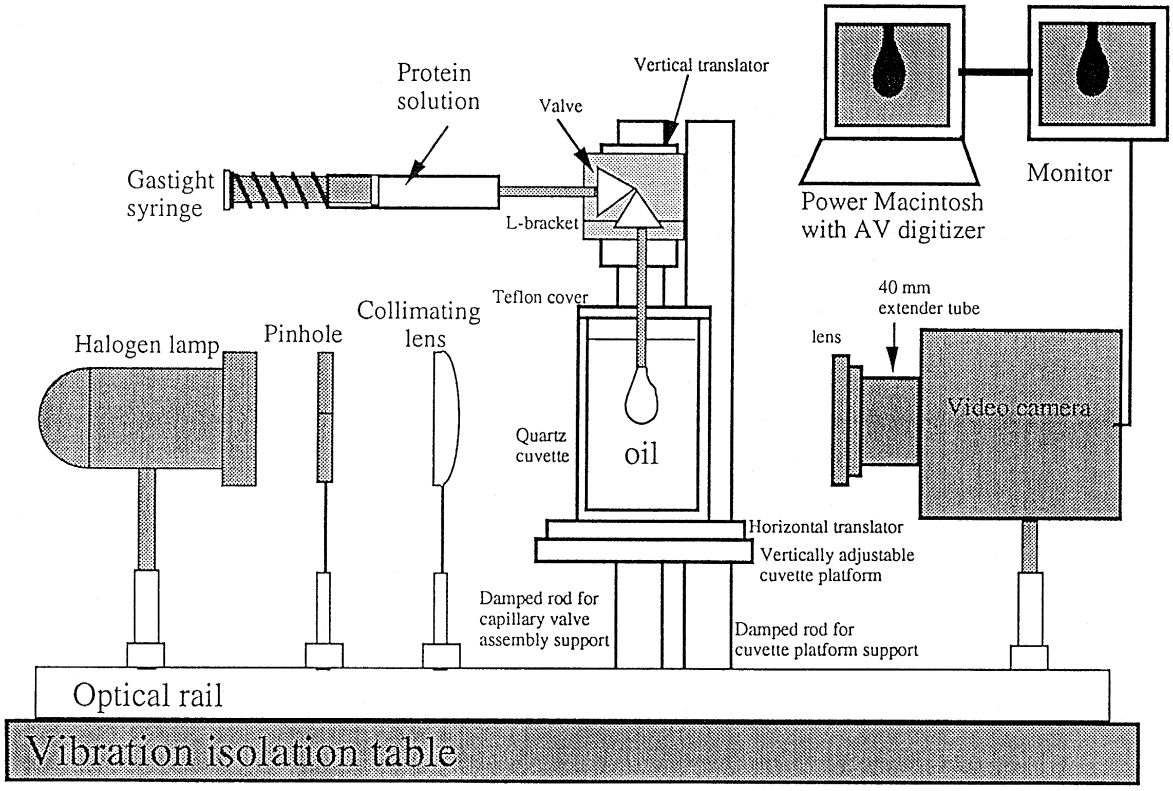

Fig. 1. Schematic of the pendant-drop apparatus.

this apparatus is shown in Fig. 1. This technique determines the interfacial tension of an oil/water boundary by the shape of a gravity-distorted liquid drop. An aqueous drop is formed and suspended from a vertical stainless steel circular capillary immersed in a water-saturated heptane phase. The resulting axisymmetry of the drop allows the shape to be described completely in two dimensions. A collimated light source illuminates the drop and surrounding heptane, and the resulting image is digitized using a CCD camera and Power Macintosh 7100 AV. The difference in indices of refraction of the heptane and water phases produces a digitized image of a dark drop silhouette (water) in a uniform, light background (heptane). This intensity difference defines the edges of the drop, and the planar drop edge coordinates are determined by the location of pixels of a minimum greyscale intensity. Sequential drop images are obtained at specified time intervals using the public-domain program NIH-Image 1.59. Programs (macros) written in NIH-Image also determine the locus of edge coordinates. These coordinates are subsequently fitted to the Young–Laplace equation, which gives the predicted profile of a pendant drop for a given interfacial tension. Results are reported as the reduction in interfacial tension from the initial value. This change is defined as the surface pressure Π:

$$\Pi(t) = \sigma_0 - \sigma(t) \quad (1)$$

where σ_0 is the interfacial tension of the pure liquid/liquid interface and $\sigma(t)$ is the dynamic interfacial tension at time t with copolymer present. The interfacial tension of a heptane/aqueous buffer system showed a reduction of 2–3 mN/m over a period of 24 h. Impurities were assumed to not affect the dynamic tensions of polypeptide solutions. Copolymer concentrations of 15 μg/ml were used for all dynamic interfacial tension measurements.

Film compression provides a simple method to observe the presence of viscoelastic interfacial layers. The pendant-drop method is particularly suitable for compression of adsorbed layers. Compression results in the collapse of adsorbed protein layers after extended exposure to an oil phase [36]. For our purposes, collapse is defined as the transition of the boundary of an illuminated pendant drop from a smooth, axisymmetric shape to an asymmetric, wrinkled shape. Compression experiments were conducted at the conclusion (18 h interface age) of the dynamic interfacial tension determinations for the synthetic glutamic acid copolymers.

Circular dichroism was measured using an Aviv Model 62DS circular dichroism spectrometer and copolymer concentrations of 40 μg/ml. The background spectrum of pure 100 mM phosphate buffer solution at the appropriate pH was subtracted. All experiments were conducted at 22 ± 1°C. Additional experimental procedures are available [37].

3. Experimental results

3.1. Copolymer adsorption and dynamic interfacial tension

Fig. 2 shows the interfacial tension as a function of time for the four glutamic acid copolymers and the homopolymer at pH 7.1. The trends at pH 7.1 indicate minimal adsorption, with maximum surface pressures of only 9 mN/m attained for any of the polypeptides. The glutamate residues (pK_a values ranging from 4.4 to 4.6) are charged at this pH, and confer aqueous solubility. Significant is the observation that at long times constant surface tension is not attained; the surface pressure continues to increase over a period of days. With air/water systems, distinct regimes in interfacial tension behavior have been identified, characterized as diffusion, conformational change, and aggregation. These have time constants that range from seconds to hours and even days [16–19]. The inability to attain an equilibrium interfacial tension is common for adsorption of globular proteins at air/water interfaces [20], and the adsorption process is believed to be irreversible [16,23,24,38,39]. The absence of an equilibrium interfacial tension for the heptane/water system is even more apparent at pH 5.3, as is illustrated in Fig. 3.

The dynamic interfacial tension of a glutamic acid homopolymer was used as a control experiment to study adsorption trends of glutamate copolymers. At pH 7.1, poly-glutamate maintains a high negative surface charge due to the ionization of the glutamate residues. The significant charge density of the homopolymer results in a preference for the aqueous phase. The affinity of the homopolymer for the

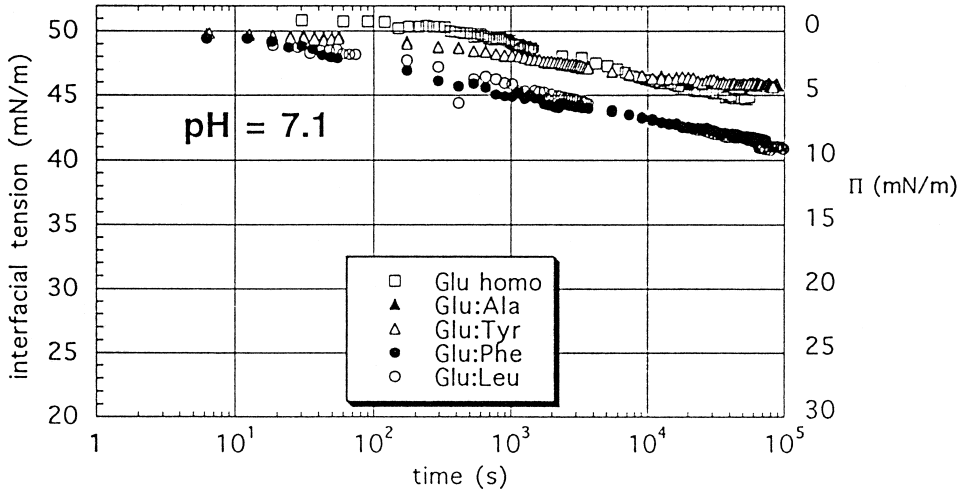

Fig. 2. Dynamic interfacial tensions of glutamic acid copolymers at pH 7.1 at the heptane/water interface. Copolymer concentrations were 15 μg/ml, ionic strength 100 mM. Surface pressures (Π, mN/m) are also indicated.

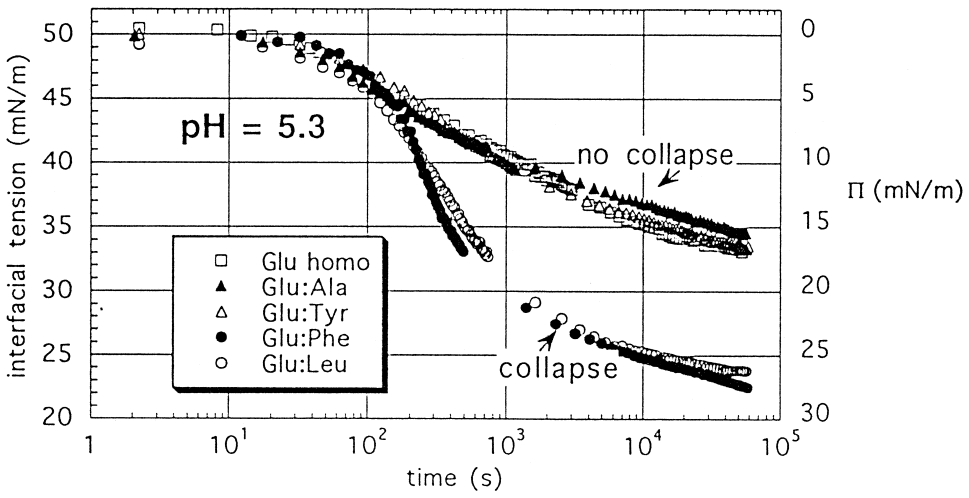

Fig. 3. Dynamic interfacial tensions of glutamic acid copolymers at pH 5.3 at the heptane/water interface. Copolymer concentrations were 15 μg/ml, ionic strength 100 mM. Collapse is defined as the visible wrinkling of the adsorbed layer upon compression of the interfacial area. Surface pressures (Π, mN/m) are also indicated.

non-polar heptane phase is not strong enough to cause significant adsorption and reduction of the interfacial tension. From Fig. 2, the small overall decrease in interfacial tension (5 mN/m) suggests minimal or very weak adsorption of the homopolymer at pH 7.1, as the decrease is only slightly larger than that observed for a pure heptane/water system.

The glu–ala copolymer is available at a mole ratio of approximately 6:4, providing a much lower surface charge than the homopolymer. The interfacial tension history shown in Fig. 2 indicates a very similar behavior between the glu–ala and glutamate homopolymers. At pH 7.1 the tension decrease is minimal. Substitution of 42% alanine groups is not sufficient to overcome the preference of the charged residues to remain in the aqueous phase. A region containing multiple alanine residues may be able to adsorb to the heptane phase, but such regions are unlikely to occur in a 6:4 random copolymer.

In contrast to alanine, the effects of the length of an aliphatic side chain can be seen in the leucine copolymer data. Fig. 2 shows the dynamic interfacial tension of a glu–leu (85:15) copolymer. At pH 7.1, the tension decrease over a period of 28 h is approximately 9 mN/m. This indicates that although the majority of glutamate residues is charged, leucine side chains are able to penetrate the interface and create a larger tension reduction compared to the homopolymer. Although the glu–leu ratio in the peptide is 4:1, remarkably, leucine maintains a sufficiently high affinity for the heptane phase to rearrange itself and the adjacent residues in such a way as to increase the surface pressure of the adsorbed film.

Phenylalanine was used to examine the effects of an aromatic side chain on interfacial affinity. The copolymer employed had a low degree of phenylalanine incorporation (81:19), similar to the glu–leu copolymer. The trend at pH 7.1 is similar to that observed for the glu–leu system. A tension decrease of 9 mN/m implies that the aromatic ring is sufficient to increase the heptane-phase affinity despite the surface charge density of the copolymer.

The tyrosine copolymer was used to investigate further the nature of the side chain on interfacial tension. The high peptide charge at pH 7.1 hinders or possibly prevents adsorption, as indicated by the small reduction in interfacial tension of 4 mN/m. This result is similar to the control case and glu–ala, indicating an analogous weak interfacial attraction in the glu–tyr system.

As illustrated in Fig. 3, larger interfacial tension changes are seen at pH 5.3, and the interfacial tension continues to decrease with time for all copolymers. A greater proportion of glutamate side chains is protonated. This protonation reduces the

dominant charge effects seen at higher pH, resulting in greater hydrophobicity and surface activity of all of the poly-amino acids. The greater number of adsorbed residues at pH 5.3 is reflected in the larger reductions in interfacial tension, as shown in Fig. 3. Here, the lower pH of 5.3 results in a tension decrease of approximately 18 mN/m for the glutamic acid homopolymer. This is an indication of a stronger tendency of polymer segments to adsorb. Glutamic acid side chains are able to either penetrate the oil phase or lie close to the interfacial region.

At pH 5.3, the glu–ala system exhibits a tension reduction of 16 mN/m that slowly increases at long times. Similarities with the homopolymer case suggest the influence of the charge of the glutamate dominates the dynamics of tension reduction. Alanine does not augment the surface pressure of the adsorbed film, as might be expected by the relatively low hydrophobicity of a one-carbon side chain.

The long-time glu–leu tension reduction is approximately 26 mN/m at pH 5.3, a value 8–9 mN/m lower than the control homopolymer at this pH and clearly indicates a polymer structural effect. The reduction of the surface charge allows more residues to approach or penetrate into the heptane phase, thereby increasing the long-time surface pressure and effectively enhancing the amount and rate of adsorption. In this case, the hydrophobicity of the leucine residues is sufficient, even at low incorporation levels, to overcome the charged glutamic acid residue effects.

A significant tension decrease is also seen in the glu–phe system at pH 5.3, closer to the pK_a of the glutamate carboxyl group. This is indicative of the strong adsorption that occurs as the phenyl groups penetrate the heptane phase, governed by dispersion and hydrophobic forces. Surface pressures close to 30 mN/m are obtained after many hours, with a slow reduction still apparent at very long times.

At pH 5.3, the interfacial tension dynamics of the tyrosine copolymer again are comparable to the low-hydrophobicity systems, with surface pressures of 16 mN/m attained at long times. Based on this result, residue size and hydrophobicity do not appear to be the sole factors determining ability to reduce interfacial tension during adsorption. The hydrophobicity of tyrosine is of the same magnitude as that of leucine and phenylalanine, although the two copolymers (glu–leu, glu–phe) exhibit higher final surface pressures at both pH values. The fact that the tension history of the glu–tyr system resembles the low hydrophobicity systems (glutamate homopolymer, glu–ala) appears to be due to the hydroxyl group of tyrosine. It appears that in order for these systems to adsorb at the heptane/water interface, hydrophobic groups must not only overcome the charge density of the glutamate residues, but include a side group that is readily solvated in the heptane phase. The hydroxyl group of tyrosine in proteins is ionized around pH 10, and hence this residue is not charged at the pHs studied here. The hydroxyl group of tyrosine thus appears to prevent any increase in surface pressure that may otherwise result from the hydrophobicity of this side chain.

3.2. Interfacial area compression

Film compression provides a simple method to observe the presence of viscoelastic interfacial layers. The similarities in structure and dynamic interfacial tension trends at the heptane/water interface between globular proteins [37] and some of the poly-amino acids studied here suggest that these copolymers are also able to form interfacial films. To examine the mechanical strength of these films, area compression was performed at 18 h interfacial age. By slowly removing volume from pendant drops of the oil-adsorbed copolymers (5–7 μl/s), the corresponding reduction in area caused the collapse of some adsorbed films, as noted in Fig. 3. Visible collapse was seen as a wrinkling of the 'skin' that had formed at the heptane/water boundary. Fig. 4 illustrates the behavior of the drop during compression tests. Visible collapse is used here as a measure of the strength of an interfacial polypeptide network, and we propose that those films, which exhibit collapse upon compression, have greater strength and thus greater film-stabilizing intermolecular attractions. The visible film collapse and the ability to collect gelatinous material from the interface also suggest that the adsorbed film is of more than molecular thickness.

Compression experiments of adsorbed poly-amino acids at pH 7.1 reveals no collapse for any of the copolymers. This observation is consistent with the above results that suggest weak adsorption of poly-

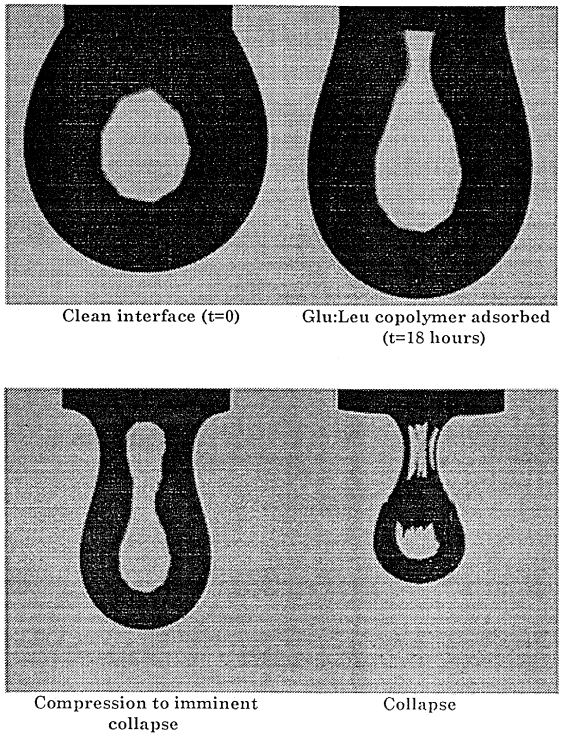

Fig. 4. Progression of a pendant-drop profile during copolymer adsorption at the heptane/water (buffer) interface. The drop contains 15 μg/ml aqueous glu:leu solution at pH 5.3, and the external phase is heptane. Upper pictures (a) and (b) show interfacial tension reduction over the course of an experiment. Reducing the volume of the drop (c) compresses the copolymer layer at the interface and results in a wrinkled skin being formed (d).

peptides occurs at the heptane/water interface in solutions above the pK_a of the carboxylate group of glutamate. The negatively charged glutamate residues appear to lower the affinity of the polypeptides for the interface as well as inhibit the development of lateral interactions believed to be necessary for the formation of a gel-like interfacial network.

At pH 5.3, compression of the adsorbed films yields results that confirm the trends seen with dynamic interfacial tension measurements. For polyglutamic acid, glu–ala, and glu–tyr, the adsorbed interfacial films appear gel-like upon compression, but do not collapse, even under high degrees of compression, as indicated on Fig. 3. A significant quantity of polymer is adsorbed, consistent with the 15–17 mN/m surface pressures seen in the dynamic tension data. The inability to form a wrinkled skin suggests the interactions between side chains of these copolymers are not as strong as those in proteins, which do form a collapsible film at the heptane/water interface [37]. These synthetic polypeptides may either desorb from the interface upon compression, form a highly flexible network that is not macroscopically evident, or form a weak network in which area compression overcomes any film-stabilizing forces. This behavior distinguishes these polypeptide systems from that of many globular proteins [37].

As indicated in Fig. 3, only the glu–leu and glu–phe copolymers adsorbed at the heptane/water interface exhibit collapse at pH 5.3. The large reduction in interfacial tension appears to correlate with the ability of leucine and phenylalanine to form strong, viscoelastic films. The lower pH reduces the surface charge of the molecules, allowing favourable hydrophobic interactions of nonpolar residues with the heptane phase and with each other. The effect of the oil phase allows multiple layers of molecules to aggregate and form a macroscopically visible gel structure. It is apparent that formation of such a structure is contingent on interactions between the hydrophobic residues. The ability to form a strong skin is remarkable, since only about 20% of the polymer incorporate these residues.

3.3. Copolymer conformation

The dynamic tension results for the glutamic acid copolymers indicate that adsorption kinetics strongly

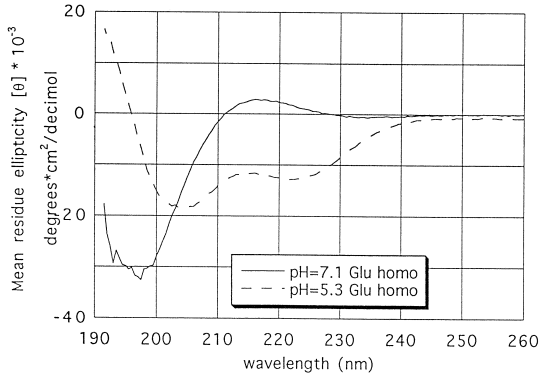

Fig. 5. Circular dichroism spectra of poly-glutamic acid (homopolymer) at pH 7.1 and pH 5.3.

depend on pH. Accordingly, it is likely that the bulk structures of these systems are also pH dependent. Changes in pH may play a role in altering the conformation of these polymers in solution. This would lead to different structures approaching the interface, and, thus, would suggest noticeably different adsorption dynamics. Circular dichroism was employed here to determine the extent and type of structure present in the glutamic acid copolymers as a function of pH.

The CD spectra at pH 7.1 and 5.3 for the glutamic acid homopolymer are shown in Fig. 5. At the higher pH, the polypeptide exists in a predominantly random coil configuration, in agreement with recent work by Hayakawa et al. [40]. The high negative surface charge at pH 7.1 causes a significant intrachain electrostatic repulsion between glutamate residues. This effect, coupled with the affinity of the charged species for a high-dielectric environment, inhibits the polypeptide from adopting secondary structures. Helices and sheets form by intrachain hydrogen bonding of water-shielded amine and carbonyl groups. It appears that the tendency of the charged, hydrophillic side chains to remain in the aqueous phase is the dominant effect at the higher pH, causing the polypeptide to be unable to shield these backbone groups and thus inhibiting secondary structure formation.

A transition in secondary structure is seen in the polyglutamate CD spectrum at pH 5.3. The two minima indicate the presence of a predominantly α-helical structure for the glutamic acid homopolymer. This is also in agreement with the results of Hayakawa et al. [40]. A reduction of pH decreases the surface charge by protonating glutamate residues. The lowered charge enables the polypeptide to adopt a more close-packed conformation, leading to significantly enhanced secondary structure.

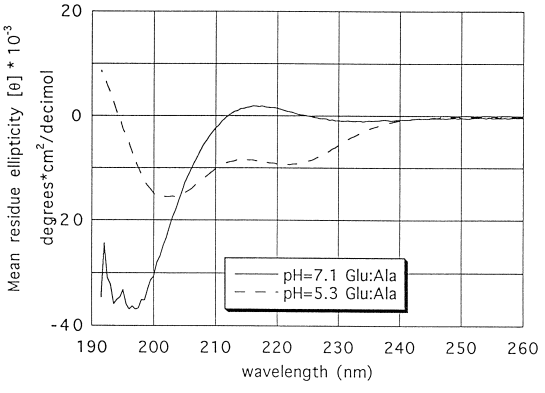

Fig. 6. Circular dichroism spectra of poly-glutamic acid:alanine (glu:ala) copolymer at pH 7.1 and pH 5.3.

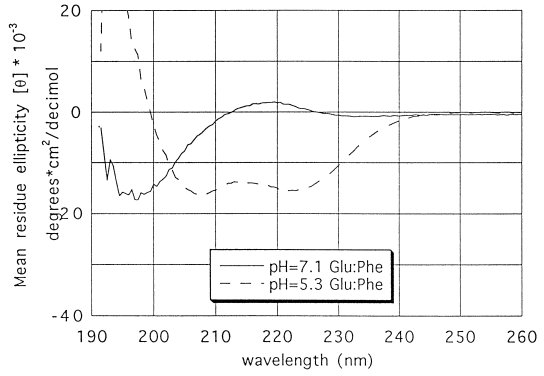

Fig. 7. Circular dichroism spectra of poly-glutamic acid:phenylalanine (glu:phe) copolymer at pH 7.1 and pH 5.3.

Fig. 6 shows the CD spectra of the glu–ala copolymer at pH 7.1 and 5.3. This polypeptide shows similar conformational trends to those of the homopolymer. High pH promotes a random coil structure, whereas lower pH allows the copolymer to adopt an α-helical structure. Incorporation of 42% alanine residues has no significant effect on the bulk structure of the peptide. The short alanine side chain causes minimal steric interference in the interactions of longer, charged glutamate residues. Thus, even at a lower percentage of glutamate in the polymer, it appears that glu–glu interactions dictate the conformation of this copolymer.

The glu–phe CD spectra are shown in Fig. 7. Similar results are seen for this system also, with neutral pH promoting a random coil structure and lower pH favouring an α-helical conformation. The contribution of phenylalanine is small, although the incorporation ratio in this copolymer is lower (19:81) compared to glu–ala (42:58). The hydrophobic phenylalanine residues contribute to the apolar environment needed for development of secondary structure.

The similarities seen in the circular dichroism spectra demonstrate that glutamate residues dictate the bulk structure of the polymers. The copolymers

studied here have a helix-coil transition as pH increases above pH 5.3. The effects of the substituted amino acid appear to be minimal, with copolymers of small side chains (ala) displaying the same trends as bulky, hydrophobic side chains (phe).

4. Discussion

Our results on the adsorption of synthetic poly-amino acids at the heptane/water interface enable several observations to be made on the driving forces and interactions among individual side chains during the adsorption process. As with dilute protein solutions, the interfacial tension dynamics of polypeptides exhibit an induction period characteristic of that found with proteins at low concentrations, where weakly adsorbing molecules arrive by diffusion at the interface but do not noticeably decrease the interfacial tension. Following this lag period, decreases in interfacial tension are attributed to saturation of the interface and conformational changes of the adsorbed molecules. These conformational changes increase the number of interfacial contacts per protein molecule, thereby increasing the effective concentration of adsorbed segments and lowering the tension. In some cases, a third regime is seen where slow relaxation of the adsorbed layer is believed to be important. Multilayer formation and gelation of the interfacial layer may also be significant at long times, depending on the nature of the polypeptide and the aqueous phase pH. Further details of these regimes are given elsewhere [37,19,20] for protein adsorption at oil/water and air/water interfaces. Changes in interfacial tension continue for all polypeptides studied even after 2 days with little evidence of an equilibrium tension.

Polymers composed chiefly of glutamic acid are highly charged in aqueous solution at pH 7.1, with predominantly random coil structures. Although minimal decreases in tension are seen for all five poly-amino acids studied here, a trend attributable to side chain structure is apparent. Long-time surface pressures of 4–5 mN/m are seen for the glutamate homopolymer, glu–ala, and glu–tyr copolymers. The glu–leu and glu–phe copolymers reach surface pressures of 8–9 mN/m. At pH 7.1, the effects of hydrophobicity and structure of the second amino acid are apparent, as indicated by this difference in pressure. The polypeptide is able to arrange itself so that the leucine and phenylalanine groups encounter the heptane phase. The two opposing effects of peptide charge interactions (glu dominated) and hydrophobic interactions (leu, phe/heptane phase dominated) appear to determine the dynamics of interfacial tension reduction. At pH 7.1, the charge effects are dominant.

At pH 5.3, the poly-amino acids are closer to the isoelectric point of glutamic acid and hence are less charged. The effect of their increased adsorption to the heptane interface is apparent, as the interfacial tension is lowered for all copolymers compared to values at pH 7.1. Glutamic acid homopolymers, glu–ala, and glu–tyr all exhibit similar rates of tension reduction and ultimate surface pressures of approximately 16–19 mN/m. The dynamic tension data reveal an induction period at early times, but do not exhibit distinct regimes. This is due to the weaker adsorption and gelation tendencies of the residues in the glutamate homopolymer, glu–ala, and glu–tyr copolymers.

Glu–leu and glu–phe show different behavior at pH 5.3, with interfacial tension dynamics similar to those for globular proteins [37]. Three time scales (induction, monolayer saturation, and gelation), characterized by slope changes on tension/log time plots, are observed during protein adsorption, and these are also seen with the leucine and phenylalanine copolymers. Interfacial tension reduction for these copolymers is approximately 27 mN/m after 18 h. The reduced charge at pH 5.3 allows the effects of the second amino acid structure on adsorption to be seen. Hydrophobic residues strongly adsorb (e.g., leu, phe). However, the hydroxyl group of tyrosine reduces adsorption. Short chains (ala) and charged or polar (homo-glu) residues also have comparatively low affinities for the heptane interface. At low pH, the competition between charge and hydrophobic effects favours the latter, with amino acid structure clearly influencing the adsorption dynamics by permitting the residues to seek a preferred liquid phase.

Polymer charge plays a major role in adsorption and the corresponding tension reduction at the heptane/water interface. Copolymers of glutamic acid become increasingly charged as the pH increases from the isoelectric point of 4.5 of the carboxyl

residue. For all the systems studied, the dynamic interfacial tension behavior and low ultimate surface pressures at long times (18 h) indicate minimal adsorption at pH 7.1. The high surface charge at this pH reduces the solvation of the polymer in the oil phase. At pH 5.3, however, the negative charge density of the polypeptides is reduced, increasing contact of hydrophobic residues with the interface. As a result, dynamic tensions characteristic of increased adsorption are seen. Surface pressures attain much higher final values of 16–28 mN/m at the lower pH. Charge effects are also noted in the circular dichroism spectra of these copolymers. At pH 7.1, these polypeptides exist in a random coil configuration in the bulk aqueous phase. The charges on glutamate residues are significant and destabilize any secondary structures. At lower pH, increased secondary structure develops in the form of α-helices due to charge reduction.

Based on the tension and CD results, glutamic acid copolymers able to form compact secondary structures in the bulk (pH 5.3) appear to have a greater affinity for the heptane interface, whereas those in random conformations are less likely to increase the surface pressure. Although the hydrophobic regions are mainly located at the interior of a protein, most globular proteins strongly adsorb at the heptane–water interface [37], indicating that small hydrophobic surface regions may be sufficient to initiate adsorption. Thus, the observation that the synthetic poly-amino acids studied here display a correlation between the presence of secondary structure and a reduction of interfacial tension follows that observed with proteins. Conversely, at pH 7.1, the increased negative charge results in both a random coil conformation and a preference for the polypeptide to remain in the high dielectric aqueous medium. In this case, charged groups screen most effects of the hydrophobic amino acids. Destabilization of secondary structure occurs via repulsive coulombic interactions. Although the random coil is in a more flexible conformation, the charged groups do not permit significant hydrophobic interactions between nonpolar residues and the oil phase. Thus, adsorption is limited, and changes in surface pressure are small.

The structure and hydrophobicity of the various side chains for the glutamic acid copolymers are also important in determining the properties of long time gel-like interfacial layers. Compression of the interfacial area for the five systems at pH 7.1 shows no tendency of the adsorbed layer to collapse as a wrinkled skin. This indicates that only minimal adsorption occurs at high pH. At pH 5.3, area compression results in collapse only for films formed from glu–leu and glu–phe copolymers. This distinction suggests that contact between bulky, hydrophobic residues promotes the formation of a gel network. Side chain length is also a factor. Short alanine side chains are unable to interact strongly (also due to low hydrophobicity) when compared to longer chains like leucine. The terminus of the side chain also plays a role as can be seen by comparing the glu–phe and glu–tyr copolymer results. Structurally, the only difference between the two residues is an additional *para*-hydroxyl group in tyrosine. The difference in amino acid hydrophobicity from such an addition is shown in Table 1 to be small. Phenylalanine, when incorporated into a glutamic acid copolymer, reaches high surface pressures and a collapsible gel layer at the heptane/water interface, whereas tyrosine reaches lower pressures and does not collapse. Thus, the difference seen in adsorption behavior of these otherwise equal systems is attributed to the hydroxyl group. The hydroxyl moiety does not permit the degree of interfacial contact that copolymers of phenylalanine enjoy, and also appears to prevent intimate contact of the aromatic rings, which may provide the strength seen in the collapsible glu–phe film.

The relationship between tension reduction and interfacial skin formation appears to be direct: large tension reductions correlate with strong gel layers at the heptane/water interface. We separate the two effects for purposes of clarity. At short times, reduction in tension is caused by the presence of adsorbed residues in the interface. A large number of penetrating residues cause a large decrease in the tension. Over time, the adsorbed molecules form a viscoelastic film or skin at the interface. Subsequent changes in interfacial tension may result from the development of a gel network and the conformational relaxation of adsorbed molecules with less dependence on number of oil-penetrating residues. The transition from a liquid/liquid boundary to a polymer network is difficult to characterize. Based on interfacial ten-

sion measurements, it is not possible to specify when this transition occurs.

5. Conclusions

Adsorption of copolymers of glutamic acid, as measured by dynamic interfacial tension at a heptane/water interface, shows a dependence on side chain structure. Copolymers incorporated with large nonpolar residues lower the tension at the heptane/water interface more than less hydrophobic residues. Interfacial tensions do not reach equilibrium and show a continued reduction. The nature of the side chain affects tension dynamics, with tyrosine copolymers displaying lower tension reduction than phenylalanine copolymers. Reduction of polymer charge by protonation at low pH enhances the adsorption of all the glutamate polymers studied here, with leucine and phenylalanine copolymers forming viscoelastic skins at long times. The pH also determines the bulk secondary structure of the copolymer. Glutamic acid copolymers are useful models for examining adsorption.

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