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The Surface-Active Properties of Muscle Proteins

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ABSTRACT

The surface-active properties of myosin, actomyosin, F- and G-actin at the air–solvent interface, at initial bulk phase concentrations in the range of 10^{-4}% to 10^{-2}% w/v, were determined by the drop volume method. Overall myosin was the most effective surface tension depressor, followed by actomyosin, F-actin and G-actin. The surface pressures attained after 40 min at an initial bulk phase concentration of 10^{-2}% w/v were 21·02, 19·27, 17·25 and 14·91 mNm^{-1}, respectively. Furthermore, myosin effected the most rapid change in surface pressure during the initial 5-min period.

INTRODUCTION

Adsorption of proteins at liquid interfaces and conformational changes following adsorption play a crucial role in the formation and stability of many food emulsions (MacRitchie, 1978; Dickinson & Stainsby, 1982; Kinsella, 1984; Dickinson, 1987). The adsorption properties of lysozyme, β-casein, bovine serum albumin and β-lactoglobulin have been widely studied with the view of defining the chemical and structural characteristics of proteins that affect their interfacial and film-forming properties. Despite their importance in meat emulsion formation (Jones & Mandigo, 1982; Jones, 1984), little work has been done on the surface properties of isolated muscle proteins at air–solvent or oil–solvent interfaces. Recent studies have shown that myosin binds strongly at the surface of controlled-pore glass

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granules, which have been chemically modified, to produce a hydrophobic surface analogous to fat particles in an emulsion (Brock, 1987; Brock & Enser, 1987). Myosin is considered to be as surface-active as casein and much more surface-active than gelatin (Dickinson *et al.*, 1987). Preliminary data from our laboratory (Morrissey *et al.*, 1987) also suggest that myosin is considerably more surface-active than actin.

The objective of the present study was to define more clearly the adsorption characteristics of myosin, actomyosin and actin at air–solvent interfaces and to discuss the probable contribution of the two main myofibrillar proteins, viz. myosin and actin, to emulsion formation and stabilization.

MATERIALS AND METHODS

All the chemicals used were of reagent grade. The water used for surface activity measurements and protein preparation was twice distilled in a glass apparatus. All the glassware used was allowed to stand overnight in sulphuric acid/dichromate solution and then rinsed several times with double-distilled water. Dialysis tubing was washed first with 1% acetic acid, rinsed with 1% Na_2CO_3 solution containing 1 mM EDTA and then heated at 75°C for 10 min in the latter solution. The tubing was rinsed with double-distilled water and again heated at 75°C in double-distilled water and rinsed several times with double-distilled water prior to use.

Determination of protein content

Protein content of all solutions were determined by the Kjeldahl method (AOAC, 1975).

Preparation of muscle protein solutions

Rabbit skeletal muscle was used in all studies. Myosin was prepared by the method outlined by Margossian and Lowey (1982). In order to remove traces of C-protein and actin, myosin was further purified by chromatography on DEAE-Sephadex using a linear KCl gradient (0 to 0·5M KCl in phosphate buffer, pH 7·5). The myosin-containing fractions were pooled and exhaustively dialysed at 4°C against 0·6M KCl, pH 7·0. F-actin was prepared by the procedure outlined by Pardee and Spudich (1982*a*). The pellets of F-actin were gently resuspended in cold 10 mM imidazole buffer, pH 7·0, containing 0·1M KCl and 0·5 mM dithiothreitol, and were dialysed overnight against the same buffer. The F-actin solution was then dialysed exhaustively

against 0·1M KCl, 10 mM ATP, pH 7·0. The G-actin fraction was prepared by resuspending F-actin pellets in cold 2 mM Tris-HCl buffer, pH 8·5, containing 0·2 mM ATP, 0·5 mM dithiothreitol and 0·1 mM $CaCl_2$, and dialysing against 10 volumes of this buffer with two changes over a 3-day period. The depolymerized actin solution was centrifuged at 80 000*g* for 3 h and subsequently dialysed against distilled water. Actomyosin was prepared by the method of Hay *et al.* (1972) and dialysed against 0·6M KCl, pH 7·0. Purity of the preparations was determined by SDS-polyacrylamide gel electrophoresis using the method of Greaser *et al.* (1983).

Prior to surface activity measurement the protein solutions were centrifuged at 20 000*g* for 30 min and the supernatants were filtered through Whatman No. 1 filter paper to remove any protein particles that could sediment during the surface tension measurements. The protein contents of the filtrates were determined and appropriate concentrations (10^{-2}% to 10^{-4}% (w/v)) prepared by dilution using the relevant solvent. The protein solutions were adjusted to pH 7·0 using 0·2M NaOH or 0·2M HCl. Surface tension measurements were made immediately after protein preparation.

Surface tension measurements

The surface activities of the protein solutions at the air–solvent interface at 25°C were determined by the drop volume principle using the apparatus described by Tornberg (1977). Calibration of the drop volume apparatus and calculation of surface tension were carried out as described by Tornberg (1978*a*) and Arnebrant and Nylander (1985). The protein solutions were prepared in triplicate and measurements of surface tension at each drop size were performed in quadruplicate. The mean surface tensions were plotted as a function of time.

RESULTS AND DISCUSSION

The time dependence of surface tension of the myofibrillar proteins, G-actin (in water, pH 7·0), F-actin (in 0·1M KCl, 10 mM ATP, pH 7·0), myosin and actomyosin (both in 0·6M KCl, pH 7·0) at the air–solvent interface at 25°C at different bulk phase concentrations are shown in Figs 1–4. The initial surface tensions (γ_0), which correspond to the surface tensions of the solvents, were 71·91, 72·25 and 73·27 mNm^{-1} for distilled water, 0·1M KCl containing 10 mM ATP and 0·6M KCl, respectively. During the time course of the experiments, surface tension (γ) was not affected by the lowest bulk phase concentration (10^{-4}% w/v) used. At a bulk phase concentration of 10^{-3}% (w/v) an initial induction period was observed where negligible changes in γ

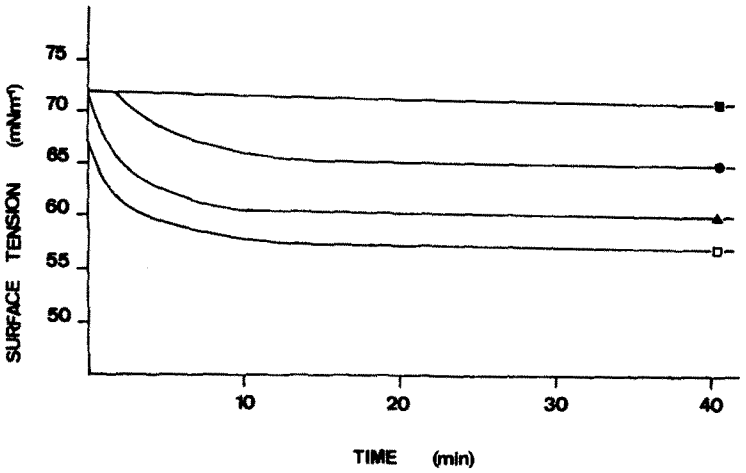

Fig. 1. Time dependence of surface tension at the air–solvent interface, at 25°C, of G-actin at bulk phase concentrations of 1×10^{-4} (■), 1×10^{-3} (●), 5×10^{-3} (▲) and 1×10^{-2} (□) %w/v protein.

occurred. Tornberg (1978*a*) and Ward and Regan (1980) concluded that the induction period was a consequence of the technique used to measure surface tension. However, the current hypothesis suggests that the induction period is due to the time taken for the adsorbing protein molecules to attain a sufficient surface coverage to decrease γ (De Feijter & Benjamins, 1987). Induction phases were observed for actomyosin at all protein concentrations studied, for F-actin at 5 and 1×10^{-3}% (w/v) and for G-actin and

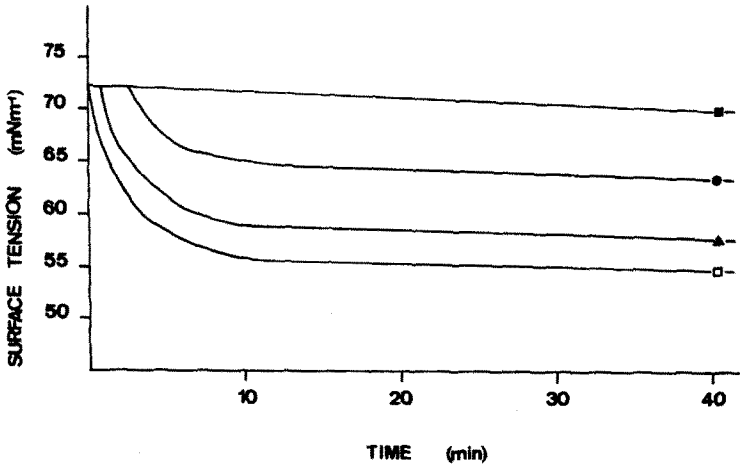

Fig. 2. Time dependence of surface tension at the air–solvent interface, at 25°C, of F-actin at bulk phase concentrations of 1×10^{-4} (■), 1×10^{-3} (●), 5×10^{-3} (▲) and 1×10^{-2} (□) %w/v protein.

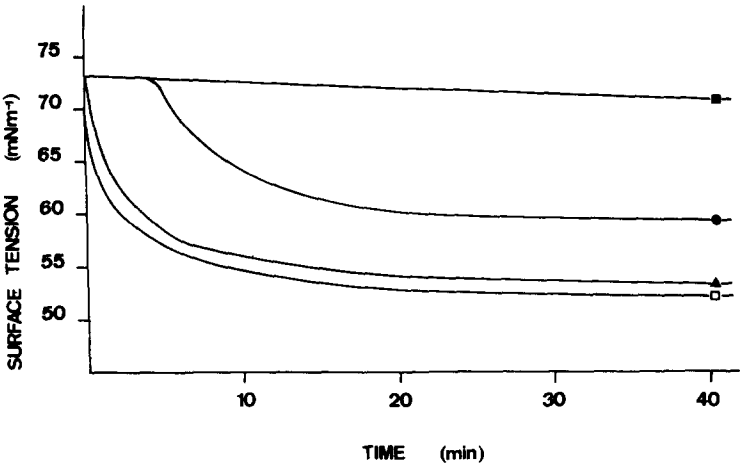

Fig. 3. Time dependence of surface tension at the air–solvent interface, at 25°C, of myosin at bulk phase concentrations of 1×10^{-4} (■), 1×10^{-3} (●), 5×10^{-3} (▲) and 1×10^{-2} (□) %w/v protein.

myosin at 10^{-3}% (w/v). The differences observed in induction times may reflect the relative rates of mass transport of the proteins to the interface.

The dependence of surface pressure on protein concentration is clearly shown in Fig. 5, where π_{40}, i.e. the reduction in surface tension at 40 min, increased as the bulk phase concentration of the proteins was increased. Overall myosin was a highly effective surface tension depressor. On the other hand, actin, and in particular G-actin, had poor surface-active properties.

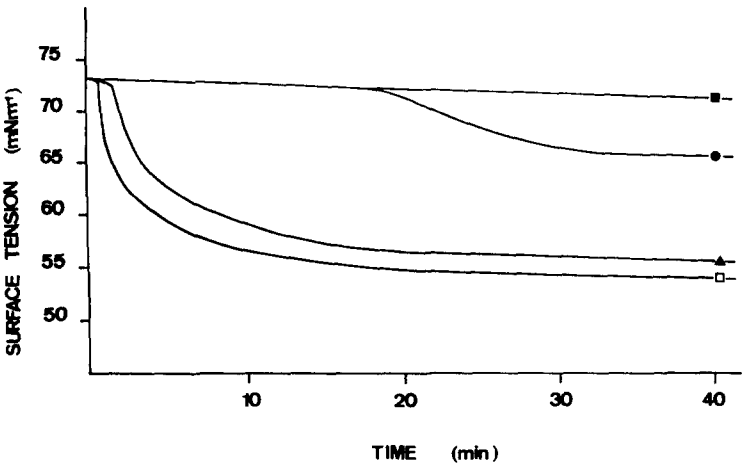

Fig. 4. Time dependence of surface tension at the air–solvent interface, at 25°C, of actomyosin at bulk phase concentrations of 1×10^{-4} (■), 1×10^{-3} (●), 5×10^{-3} (▲) and 1×10^{-2} (□) %w/v protein.

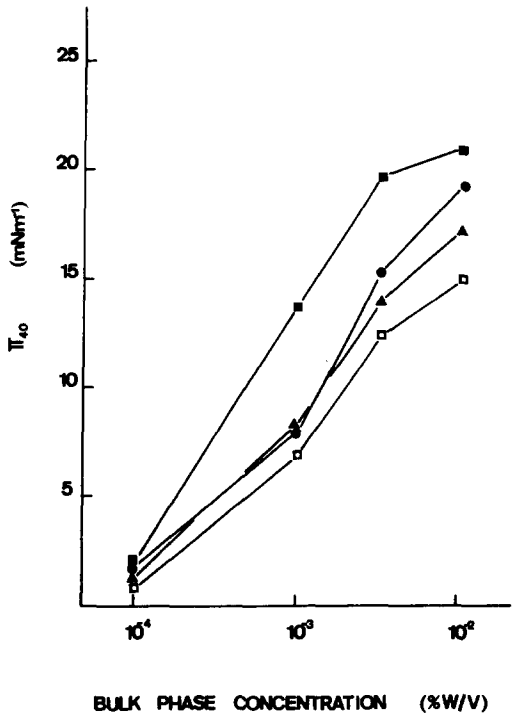

Fig. 5. The surface pressure attained after 40 min, π_{40}, as a function of the initial bulk phase protein concentration for myosin (■), actomyosin (●), F-actin (▲) and G-actin (□).

Actomyosin, which is a complex of F-actin and myosin, occupied an intermediate position between the other two proteins at high bulk phase concentrations, but exhibited similar surface activity to F-actin at low bulk phase concentrations.

Myosin is known to contain a number of surface hydrophobic sites (Borejdo, 1983) and surface hydrophobicity is reported to correlate well with surface activity (Kato & Nakai, 1980; Kato *et al.*, 1981). Thus it was not surprising to find, in the present study, that myosin was highly effective in reducing surface tension. The binding of F-actin to myosin to form actomyosin does not affect the hydrophobic effectiveness of myosin (Borejdo, 1983). The present results suggest that the surface activity of myosin was not adversely affected by complexation either, and it is probable that the observed reduction in the surface activity of actomyosin, compared with myosin, was due to the reduced myosin concentration rather than complexation.

It is apparent from the present data that actin was not as surface-active as myosin. These results, in essence, support the findings of Brock and Enser

(1987), who showed that G-actin did not bind at the surface of controlled-pore glass beads which had been chemically modified to produce a hydrophobic surface, and concluded that actin has a strongly polar surface and that the hydrophobic amino acids, which occur predominantly in long apolar amino acid sequences (120–153, 293–305 and 336–357) (Collins & Elzinga, 1975), are buried in the interior of the globular molecule and inaccessible to the hydrophobic interface.

Polymerization of G-actin to form F-actin enhanced the surface activity of the protein (Fig. 5). Various studies have shown that conformational changes occur during the G- to F-actin transition (Tawada *et al.*, 1969; Stone *et al.*, 1970; Bridgen, 1972; Rich & Estes, 1976; Pardee & Spudich, 1982*b*). These conformational changes may be responsible for the increase in the surface activity of the polymerized protein.

A rapid reduction in interfacial tension is important in the stabilization of lipid droplets during emulsification (Halling, 1981). Thus the rate of decrease in interfacial tension or increase in surface pressure ($\Delta\pi/\Delta t$) effected by a protein is an important characteristic of its interfacial behaviour. In Table 1, $\Delta\pi/\Delta t$ during the first 30 s and 5 min are reported for the proteins at 10^{-2}%,

TABLE 1
Parameters Describing the Rates of Surface Tension Decay at the Air–Solvent Interface for Myosin, Actomyosin, F-Actin and G-Actin

Protein concentration (% *w/v*)	*Induction period* (*min*)	$\Delta\pi/\Delta t$ *0·5 min* ($mNm^{-1}\,min^{-1}$)	$\Delta\pi/\Delta t$ *5·0 min* ($mNm^{-1}\,min^{-1}$)	π_{20} (mNm^{-1})	π_{40} (mNm^{-1})
Myosin					
1×10^{-2}	—	18·04	3·25	20·27	21·02
5×10^{-3}	—	10·54	3·00	18·97	19·77
1×10^{-3}	4·66	—	0·35	12·97	13·77
Actomyosin					
1×10^{-2}	0·66	—	2·85	18·52	19·27
5×10^{-3}	1·50	—	2·15	17·02	17·77
1×10^{-3}	20·00	—	—	1·77	7·77
G-actin					
1×10^{-2}	—	14·28	2·54	14·71	14·91
5×10^{-3}	—	6·32	1·93	11·81	11·91
1×10^{-3}	1·83	—	0·72	6·71	6·91
F-actin					
1×10^{-2}	—	8·50	2·82	17·00	17·25
5×10^{-3}	0·66	—	2·15	13·75	14·00
1×10^{-3}	2·50	—	1·00	8·10	8·25

5×10^{-3}% and 10^{-3}% (w/v). The rate of change in π is considered to be a function of chemical and structural characteristics of proteins (Graham & Phillips, 1979*a–c*; Shimuzu *et al.*, 1985; Waniska & Kinsella, 1985; Song & Damodaran, 1987). Thus the different behaviour of the proteins evident from the data probably reflects the physico-chemical properties of the individual proteins. At 10^{-2}% (w/v) the rate of increase of π during the first 30 s was greatest for myosin followed by G- and F-actin. Because of the induction period very little change in π was observed for actomyosin during this time interval. We consider that $\Delta\pi/\Delta t$ during the initial 30 s reflects the rate at which the protein molecules migrate and adsorb at the interface. Thus it is not surprising that G-actin migrates faster to the interface and increases surface pressure more rapidly during the early stages of the adsorption process than the bulkier, more viscous, F-actin form.

Based on molecular size, one would expect myosin to exert a slower rate of change in π than G-actin. However, molecular flexibility and surface hydrophobicity also influence the rate of protein adsorption at the interface (Mitchell *et al.*, 1970; Graham & Phillips, 1976; Song & Damodaran, 1987; Waniska & Kinsella, 1987). Thus the greater surface hydrophobicity of myosin (Borjedo, 1983) and the elongated nature of the molecule in its native conformation (Lowey *et al.*, 1969; Squire, 1981) may be major factors contributing to its ability to effect rapid change in π. The slow development of π during the first minute and the presence of an induction period for actomyosin may be due to slow migration of the large molecular complex to the interface.

While G-actin caused a very rapid increase in π during the initial 30 s, the rate of change of π exerted by this protein subsequently decreased rapidly. The overall $\Delta\pi/\Delta t$ for G-actin during the first 5 min was lower than that for myosin, F-actin and actomyosin (Table 1). The data show that the relative effectiveness of the various proteins in increasing π during the first 5 min is a more accurate indicator of the overall surface activity of the proteins compared with $\Delta\pi/\Delta t$ for the initial 30 s period. Molecular flexibility is an important factor determining the surface activity of proteins (Graham & Phillips, 1976, 1979*a–c*; Kato *et al.*, 1985, 1986). G-actin is a rigid globular molecule with a diameter of 5 nm (Moore *et al.*, 1970). Thus the relatively small molecular size of G-actin favours its rapid adsorption at the interface; however, because of its compact globular nature it is unlikely to denature and unfold readily once adsorbed. This lack of flexibility may account for the slow rate of change of π during the first 5 min for G-actin and the overall low surface activity of the protein. In this respect, the behaviour of G-actin is probably similar to that of lysozyme (Graham & Phillips, 1976, 1979*a–c*). Conformational changes that occur during the G- to F-actin transformation

appear to confer increased flexibility to the structure of the protein, enabling it to spread more easily on the interface and give a more rapid change in π during the initial 5 min.

After 20 min, changes in π were very small for all the proteins (Table 1), and probably reflect further minor rearrangements and reorientations of the adsorbed protein molecules with little, if any, adsorption of new protein molecules from the bulk phase (Graham & Phillips, 1979*b*).

One might expect the duration of the induction period to be appreciably longer for polymerized actin than for the monomeric form at low bulk phase concentrations. However, our results show that at 10^{-3}% (w/v) the induction period is only slightly longer for F-actin than for G-actin (Table 1). At this low bulk phase concentration actin is probably below the critical concentration for F-actin formation (Gordon *et al.*, 1977). It is likely that the F-actin filaments, initially present in solution, may partially depolymerize on standing (Cooper & Pollard, 1982) and shorten in length. Thus, under these circumstances, rapid migration of actin to the interface may occur.

Our results show that myosin was the most surface-active of the myofibrillar proteins studied and that it had the ability to reduce surface tension rapidly during the initial stages of adsorption. Compared to surface pressure values after 40 min reported for other food proteins, its surface activity is similar to that of sodium caseinate and whey proteins, and is superior to that of soy protein, lysozyme and κ-casein (Tornberg, 1978*a*,*b*; Arnebrant & Nylander, 1985). Kato and Nakai (1980) observed a significant correlation between surface activity and emulsifying activity of a range of proteins. Thus, on the basis of the present study, it is reasonable to assume that myosin, because of its high surface activity, plays a major role in the initial stages of interfacial film formation and fat particle encapsulation during sausage manufacture. This conclusion is in accord with the findings of Galluzzo and Regenstein (1978), who showed that myosin is rapidly taken up at the fat–aqueous interface during emulsification.

On the basis of the present results the surface activity of muscle proteins may be ranked as follows:

$$\text{myosin} > \text{actomyosin} > \text{F-actin} > \text{G-actin}$$

These results are in line with the findings of Hegarty *et al.* (1963) and Galluzzo and Regenstein (1978) for emulsifying activity.

Thus myosin appears to be the key protein in meat emulsion formation and stabilization. However, at this point we can only speculate as to the structure and orientation of myosin molecules at the interface, and further studies are essential if we are to delineate accurately the surface-active portion of the myosin molecule.

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