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# Isolation and characterization of latherin, a surface-active protein from horse sweat

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A protein, latherin, with unusual surface activity was isolated from horse sweat by gel filtration and ion-exchange chromatography. The protein has a Stokes radius, determined by gel filtration, of 2.47 nm, and in the ultracentrifuge sediments as a single species with $s_{20,w}$ 2.05 S, indicating an $M_r$ of 24400. On SDS/polyacrylamide-gel electrophoresis the molecule behaves as a single peptide chain of apparent $M_r$ 20000. Latherin contains a high proportion of hydrophobic amino acids (37.2%), and the leucine content (24.5%) is exceptionally high. The unusual composition of the protein may account for apparent anomalies in the $M_r$ of latherin determined by empirical methods. Evidence indicating that latherin is responsible for much of the surface activity of horse sweat was obtained by a simple assay for surface tension and by contact-angle measurements. Latherin adsorbs very readily at hydrophobic surfaces, rendering them wettable. A possible role for latherin in thermoregulation is proposed.

## INTRODUCTION

Sweat produced by the horse has an unusually high concentration of protein, compared with that of other species (Jenkinson *et al.*, 1974), and is responsible for the lather seen on horses after heavy exercise. The function of this protein is not clear, but it has been suggested that sweat proteins might have a surfactant-like action, increasing the spreading of sweat and consequently aiding evaporation and cooling (Eckersall *et al.*, 1984).

Eckersall *et al.* (1982, 1984) isolated and partially characterized two major polypeptide fractions, H ($M_r$ 49000 by gel filtration) and L ($M_r$ 33000) from horse sweat. Both components were reported to contain carbohydrate, and fraction L had an amino acid composition unusually high in leucine.

The present paper describes a simple procedure for detecting surface activity and its use to follow the purification of a protein present in fraction L which is responsible for much of the surface activity of horse sweat. The characterization and some properties of the purified protein, for which the name latherin is suggested, are described.

## MATERIALS AND METHODS

Horse sweat stimulated by adrenaline infusion was collected from thoroughbred horses as described previously (Eckersall *et al.*, 1982) and was stored at −70 °C. After thawing, samples were centrifuged for 20 min at 4000 *g* to remove particulate material.

### Purification of latherin

Sweat proteins were purified by gel filtration of 4 ml samples on a column (1.5 cm × 92 cm) of Sephadex G-100 equilibrated and eluted with 0.25 M-NaCl/0.05 M-Tris buffer adjusted to pH 8.4 with HCl. The flow rate was 10 ml/h, and 2.5 ml fractions were collected and analysed by capillary-rise and $A_{280}$ measurements. Pooled fraction 3 (Fig. 1*a*) was stored frozen at −18 °C until further purification by ion-exchange chromatography.

Ion-exchange chromatography was done on a column (1.5 cm × 25 cm) of DEAE-Sephadex A-50 (Pharmacia) previously equilibrated with 0.05 M-NaCl/0.05 M-$NaH_2PO_4$ adjusted to pH 6.0 with NaOH. Fraction 3, isolated from 8 ml of sweat by gel filtration, was dialysed against repeated changes of the 0.05 M-NaCl/0.05 M-phosphate buffer and was then applied to the ion-exchange column at a flow rate of 10 ml/h. Elution was with a linear gradient produced by mixing 110 ml of 0.6 M-NaCl/0.05 M-phosphate buffer, pH 6.0, with 110 ml of 0.05 M-NaCl/0.05 M-phosphate buffer. Fractions were pooled as indicated in Fig. 2 and were stored frozen before rechromatography on Sephadex G-100 (Fig. 1*b*).

Reduced carboxymethylated latherin was prepared by the method of Allen (1981).

### Assay of surface activity

Capillary-rise measurements were performed with glass capillary tubes which had been washed in chromic acid, rinsed extensively in deionized double-distilled water, and dried. The diameters of capillaries were determined by weighing before and after filling with water and measuring their length. Samples (1 ml) of water, buffer or protein solution were placed in Technicon sample cups, which had been washed with pentane. A capillary was placed in the centre of the cup and the meniscus was allowed to rise with the capillary at an angle of about 45° to the vertical. The capillary was then moved back to the vertical position and supported. For a pure liquid such as water, the meniscus fell back to and remained at an equilibrium position. Meniscus height in the capillary was read by alignment, by using a mirror to eliminate parallax error, with a scale graduated in 1 mm intervals. The level of the lower meniscus could be observed directly in the same way, because the liquid in the transparent Technicon cup had an almost flat surface. Measurements were performed at 20 °C.

Capillary heights could be measured reproducibly within ±0.2 mm by this simple method. For a capillary

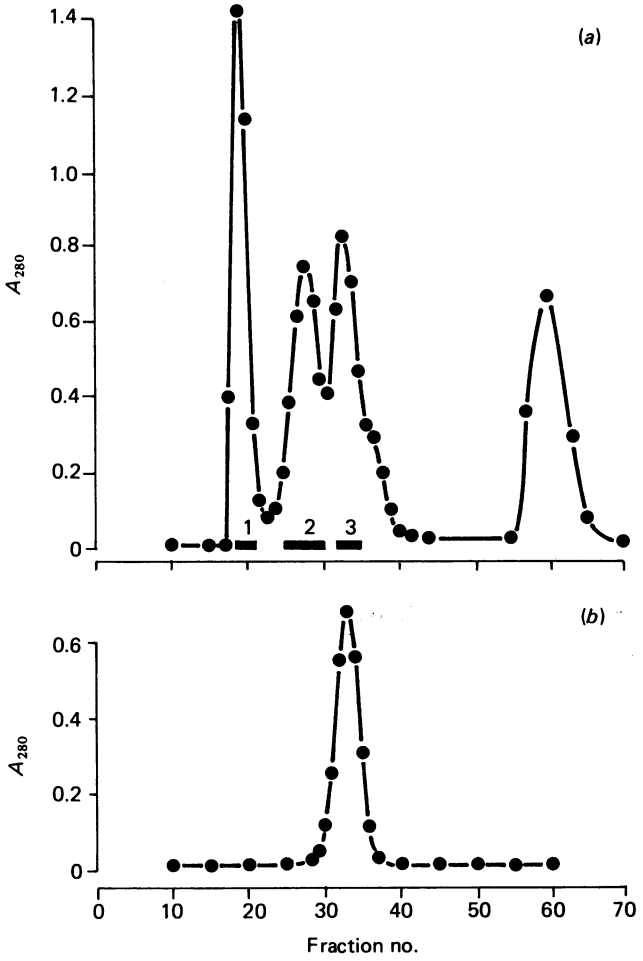


**Fig. 1. Isolation of latherin by gel filtration on Sephadex G-100**

(*a*) Elution pattern obtained when 4 ml of sweat was applied to Sephadex G-100 (1.5 cm × 92 cm) and eluted with 0.25 M-NaCl/0.05 M-Tris/HCl buffer, pH 8.4. Peaks 1–3 were pooled as indicated by the bars. (*b*) Rechromatography of peak-3 material after ion-exchange chromatography (Fig. 2). Fraction size was 2.5 ml.

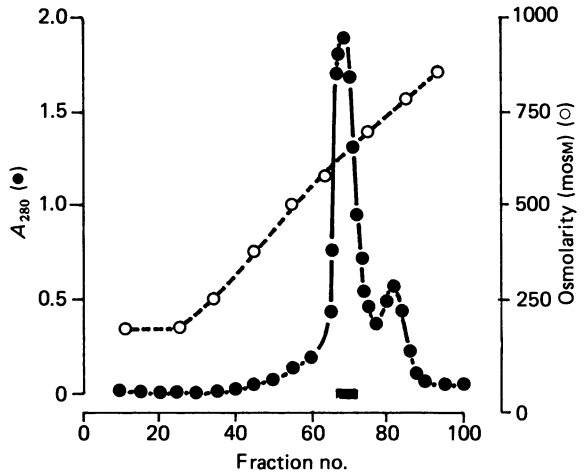


**Fig. 2. Purification of latherin on DEAE-Sephadex A-50**

The sample (peak 3 from Fig. 1*a*) was applied to DEAE-Sephadex (1.5 cm × 25 cm) in 0.05 M-NaCl/0.05 M-phosphate buffer, pH 6.0, and was eluted with a salt gradient as described in the text. Fraction size was 2.5 ml, and the pooled fractions are indicated by the bar.

of radius, 0.5605 mm a height of 26.7 mm was determined with double-distilled water, giving a value for surface tension of $0.073 \pm 0.001$ N·m$^{-1}$, consistent with the value of 0.07275 N·m$^{-1}$ obtained by more precise and elaborate methods (Adamson, 1982).

For protein solutions the capillary height was observed to change with time. An initial rapid fall in the height of the meniscus was followed by a further slow decline, continuing, for some proteins, over a period exceeding 60 min. The values for surface tension reported here were obtained from capillary-height measurements made 1 min after the meniscus was allowed to descend in the capillary. This time interval was chosen because the rate of change of meniscus height was sufficiently low for precise measurements to be made, but the contribution of slow surface denaturation and aging processes was minimized.

Advancing contact angles were measured at 20 °C by photography of 20 μl drops of buffer or protein solution applied to a horizontal surface. Measurements were made on enlarged prints by the construction method suggested by Neuman & Good (1979).

The ability of proteins to adsorb to and make hydrophobic surfaces wettable was examined with 2 cm × 1 cm pieces of Nescofilm (Nippon Shoji Kaisha, Osaka, Japan). Adsorption was carried out by suspending the Nescofilm with 1 cm$^2$ of its surface dipping into the protein solution. After 0.5 min the Nescofilm was withdrawn and any adherent liquid was removed by touching the side of the film on a filter paper. The dry Nescofilm was placed on a flat glass surface. A drop of buffer (20 μl of 0.125 M-NaCl/0.05 M-Tris/HCl, pH 8.4) was applied to the surface and was examined to determine whether it formed a rounded drop with contact angle greater than 90° (scored as −) or a partially spread drop with contact angle less than 90° (+), or the whole surface was completely wettable (++). As a control, buffer was also placed on the 1 cm$^2$ portion of the film that had not been dipped.

### Determination of $M_r$

Gel-filtration columns (Sephadex G-100) used for determination of Stokes radius and $M_r$ were calibrated with Blue Dextran (Pharmacia), bovine serum albumin (Armour), bovine α-chymotrypsinogen (Sigma), ovalbumin (Sigma) and horse heart cytochrome *c* (Sigma). $M_r$ was determined from a plot of $K_{av.}$ against $\log M_r$ (Laurent & Killander, 1964). Stokes radius was determined from a graph of $(-\log K_{av.})^{\frac{1}{2}}$ against the Stokes radii of protein standards (Siegel & Monty, 1966; Laurent & Killander, 1964).

SDS/polyacrylamide-gel electrophoresis was carried out in 15%-polyacrylamide gels (1.5 mm thick and 6 cm long), in the buffer system of Laemmli (1970). Protein standards were obtained from Pharmacia. Samples were reduced with dithiothreitol (1.6%, w/v) and heated at 100 °C for 2 min.

Sedimentation-velocity experiments were performed in a Beckman model E analytical ultracentrifuge equipped with photoelectric scanner and monochromator accessories. Protein (1 mg/ml in 0.25 M-NaCl/0.05 M-Tris/HCl buffer, pH 8.5) was centrifuged at 44000 rev./min

at 20 °C. Cells were scanned at 280 nm at 8 min intervals.

### Amino acid analysis

Protein samples, containing 0.1% (w/v) phenol and norleucine as internal standard, were hydrolysed in duplicate in 6 M-HCl for 24, 48 and 72 h in evacuated sealed tubes at 110 °C. After removal of HCl, analyses were performed on an LKB 4400 amino acid analyser (Beeley, 1985). Half-cystine was determined as cysteic acid after performic acid oxidation. Tryptophan was measured by the method of Edelhoch (1967).

### Protein determinations

Concentrations of purified latherin solutions were determined by using the $A_{1\,cm,280}^{1\%}$ of 4.72 determined by amino acid analysis of a sample of known absorbance as described by Allen (1981). The protein concentration of horse sweat was determined colorimetrically (Eckersall *et al.*, 1984).

## RESULTS

### Surface tension of whole sweat

Samples of horse sweat had pH values in the range pH 8.4–8.9, and were 535–565 mosM (Advanced Digimatic osmomometer). Accordingly, characterization of sweat proteins was carried out in solutions buffered with 0.25 M-NaCl/0.05 M-Tris adjusted to pH 8.4 with HCl (535 mosM).

Measurement of the height to which solutions rise in a capillary is a simple method for detecting surface-active molecules. The interpretation of quantitative measurements of surface tension made by this method is complicated by time-dependent changes in the surface adsorption of protein. This method therefore does not give a true equilibrium value for surface tension of protein solutions, but provides a simple, rapid, assay for the presence of surface-active molecules, which can be used to detect changes in surface activity or to monitor the purification of surface active components.

The effect of sweat proteins on apparent surface tension as measured by the capillary-rise procedure was examined. Fig. 3 shows the curve obtained when measurements were carried out on different concentrations of dialysed horse sweat. These results show that sweat contains non-diffusible molecules which markedly decrease capillary height. No difference was detected between the surface tension of dialysed and undialysed samples.

### Purification of surface-active molecules

Initial purification of sweat proteins was based on the Sephadex G-100 gel-filtration procedure of Eckersall *et al.* (1984), but with modifications of sample preparation, buffer and column dimensions which improved the resolution. Fig. 1(*a*) shows the presence of three major high-$M_r$ fractions with a shoulder on fraction 3. Capillary-rise measurements on peak fractions indicated that the breakthrough material (fraction 1) and fraction 2 had little surface activity, but tubes from the peak of fraction 3 showed greatly lowered surface tension (Table 1). The conclusion that surface-active material was concentrated in fraction 3 was reinforced by the observation that material from this fraction very readily formed a foam when drawn into a Pasteur pipette.

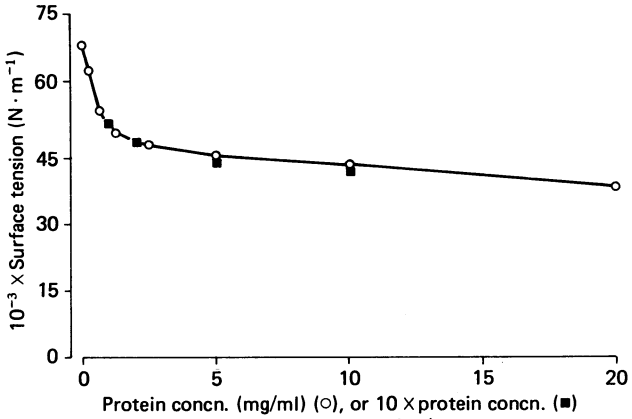


**Fig. 3. Concentration-dependence of surface tension of dialysed sweat and latherin**

Surface tension of dialysed horse sweat (○) and latherin (■) was measured by the capillary-rise method described in the text. Note the 10-fold difference in the protein concentration scales for the two preparations.

**Table 1. Surface activity of horse sweat protein fractions from Sephadex G-100 chromatography (Fig. 1)**

Values for equivalent sweat-protein concentration were obtained by interpolation of the surface tension on the curve in Fig. 3.

| Tube no. | Fraction | $A_{280}$ | Surface tension (N·m$^{-1}$) | Equivalent sweat-protein concn. (mg/ml) |
|---|---|---|---|---|
| 21 | 1 | 0.90 | 0.069 | 0.1 |
| 28 | 2 | 0.61 | 0.059 | 0.5 |
| 32 | 3 | 0.59 | 0.046 | 4.0 |
| Buffer | | | 0.073 | |

Fraction 3 was further purified by ion-exchange chromatography on DEAE-Sephadex (Fig. 2), which separated minor contaminants from the sharp major peak with which surface activity was associated. Finally, rechromatography was carried out on a newly prepared Sephadex G-100 column which had not previously been exposed to horse sweat. The major protein component of fraction 3, latherin, emerged as a single symetrical peak (Fig. 1*b*).

### Homogeneity and $M_r$

Latherin migrated as a single band on SDS/polyacrylamide-gel electrophoresis when either reduced or unreduced (Fig. 4). The reduced protein had an apparent $M_r$ of $20000 \pm 1000$, and the unreduced protein migrated with apparent $M_r$ 18000. These findings indicate the presence of a single peptide chain containing one or more intrachain disulphide linkages.

Latherin emerged from a Sephadex G-100 column with $K_{av.}$ 0.259. For a column calibrated with standard proteins, a graph of $K_{av.}$ against log $M_r$ gave a value of $M_r$ 30000 for latherin. Gel-filtration data can alternatively be used to estimate the Stokes radii of proteins. For latherin the Stokes radius determined in this way was 2.47 nm.

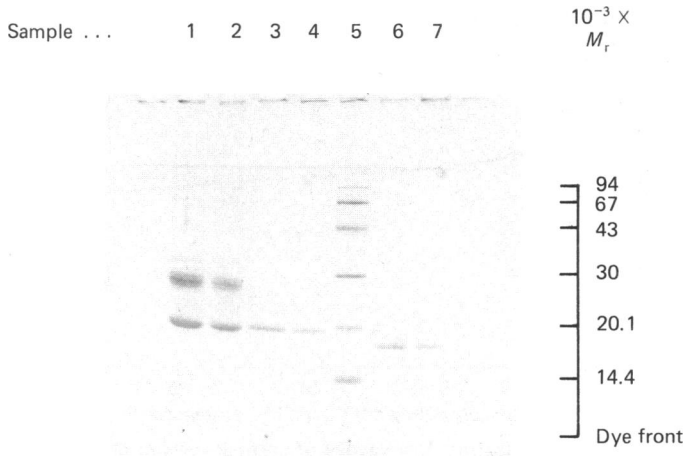


**Fig. 4. SDS/polyacrylamide-gel electrophoresis of sweat proteins**

The samples applied were: 1 and 2, horse sweat (30 and 15 $\mu$g of protein respectively); 3 and 4, purified latherin (6 and 3 $\mu$g respectively; disulphide reduced); 5, protein standards ($M_r$ values shown to the right); 6 and 7, purified latherin (6 and 3 $\mu$g; without disulphide reduction). Note in track 6 that diffusion of reducing agent from sample 5 produces a trace of reduced latherin at one side of the sample track.

Sedimentation-velocity ultracentrifugation of latherin at 44000 rev./min and 20 °C in 0.25 M-NaCl/0.015 M-Tris/HCl, pH 8.4, gave a single boundary with a sedimentation coefficient of 1.92 S ($s_{20,w}$ 2.05 S). An $M_r$ of 24400 was calculated for latherin from the sedimentation coefficient and the Stokes radius by using the relationship (Siegel & Monty, 1966):

$$M = \frac{6\pi\eta Nas^0}{1-\bar{v}\rho}$$

where $M$ is molecular weight, $\eta$ the viscosity of the medium, $N$ is Avogadro's number, $a$ the Stokes radius, $s^0$ the sedimentation coefficient, $\bar{v}$ the partial specific volume of the protein and $\rho$ the density of the medium. A partial specific volume of 0.757 ml/g was obtained from the amino acid composition by using the values of apparent specific volumes of amino acid residues tabulated by Zamyatnin (1972). As the concentration-dependence of the sedimentation coefficient was not studied, the possibility of some deviation in $M_r$ arising from this source cannot be excluded.

## Amino acid composition

The amino acid composition of latherin, corrected for destruction and incomplete release of amino acids during hydrolysis, is given in Table 2. The composition is unusual in the very high proportion of leucine, which constitutes almost one residue in four of the protein. The amino acids leucine, isoleucine and valine together account for 37.2% of the total residues. These residues have large partial specific volumes, and consequently the partial specific volume of the protein is unusually high. Latherin has a substantial content of aspartic acid and glutamic acid (26.2%).

Apart from methionine, which is absent, the amino acid present in smallest amount in latherin is histidine. A minimum $M_r$ for latherin can be calculated assuming that there is a single residue of this amino acid per molecule. An amino acid composition consistent with this assumption and with close to integral values for most other amino acids is given in Table 2. A molecule with this composition would contain 220 residues and would have $M_r$ 24200, a value close to that obtained from sedimentation and Stokes-radius data.

**Table 2. Amino acid composition of latherin**

Protein samples were analysed in duplicate after hydrolysis for 24 h, 48 h and 72 h and were corrected for destruction. Cystine was determined as cysteic acid after hydrolysis of a performic acid-oxidized sample, and tryptophan was determined by the method of Edelhoch (1967). Residues per molecule were calculated by finding the composition with best approach to integral values of residues (in parentheses).

| | Content | |
|---|---|---|
| | (residues/ 100 residues) | (residues/ molecule) |
| Aspartic acid | 14.36 | 31.4 (31) |
| Threonine | 3.92 | 8.6 (9) |
| Serine | 6.48 | 14.2 (14) |
| Glutamic acid | 11.88 | 26.0 (26) |
| Proline | 4.96 | 10.9 (11) |
| Glycine | 6.27 | 13.7 (14) |
| Alanine | 4.48 | 9.8 (10) |
| Valine | 5.52 | 12.1 (12) |
| Methionine | 0 | 0 (0) |
| Isoleucine | 7.22 | 15.8 (16) |
| Leucine | 24.50 | 53.6 (54) |
| Tyrosine | 1.37 | 3.0 (3) |
| Phenylalanine | 1.83 | 4.0 (4) |
| Histidine | 0.47 | 1.0 (1) |
| Lysine | 1.85 | 4.1 (4) |
| Arginine | 2.59 | 5.7 (6) |
| Tryptophan | 1.05 | 2.3 (2) |
| Cystine (half) | 1.23 | 2.7 (3) |
| Total ... | | 220 |

No glucosamine or galactosamine was detected in hydrolysed latherin, although standard amino sugars were well resolved from amino acids in the analytical system employed (Beeley, 1985).

## Surface activity of latherin

The surface activity of purified latherin was examined initially by measuring surface tension by the capillary-height method. Latherin (1 mg/ml) produced the same decrease in surface tension as a dilution of horse sweat containing 10 mg of protein/ml. Surface tension showed a similar dependence on protein concentration to that of horse sweat (Fig. 3). Gel scanning indicates that latherin comprises approx. 17–20% of the protein in sweat. Thus the activity of latherin could account for much of the surface-tension decrease observed in sweat.

The effect of latherin in this assay was compared with that of several other proteins (Table 3). Although there was considerable variation between proteins, latherin at 1 mg/ml was more effective at lowering capillary height than was any of the other proteins at 10 mg/ml.

**Table 3. Surface tension of latherin and other proteins**

Measurements were made on protein solutions at 1 or 10 mg of protein/ml in 0.25 M-NaCl/0.05 M-Tris/HCl, pH 8.4, by the capillary-rise method.

| | Surface tension ($N \cdot m^{-1}$) | |
|---|---|---|
| Protein concn. ... | 1 mg/ml | 10 mg/ml |
| Bovine serum albumin | 0.062 | 0.053 |
| Ovomucoid | 0.072 | 0.070 |
| Ovalbumin | 0.071 | 0.065 |
| Haemoglobin | 0.056 | 0.045 |
| Latherin | 0.042 | — |

The role of disulphide bonds in the activity of latherin was examined by measuring the surface activity after reduction and carboxymethylation. Carboxymethylated latherin (2 mg/ml) gave a capillary rise equivalent to that produced by 0.4 mg of latherin/ml indicating residual activity appreciably lower than that of the native protein.

The effect of latherin at an interface between drops of liquid and a hydrophobic surface was examined by measurement of advancing contact angle. Nescofilm was employed as the hydrophobic surface because it gives a large and reproducible contact angle with aqueous solutions and was also suitable for assessing surface wettability. At this surface 0.25 M-NaCl/0.05 M-Tris/HCl buffer, pH 8.4, had a contact angle of $98 \pm 0.5°$. The contact angle for horse sweat dialysed against this buffer (22 mg of protein/ml) was $89 \pm 1°$ and that of latherin (1 mg/ml) was $86 \pm 1°$. Thus latherin was effective in decreasing the contact angle of the buffered salt solution to a greater extent than was sweat with a 20-fold higher protein content.

Investigation of the capacity of latherin and other proteins to adsorb to, and increase the wettability of, a hydrophobic surface gave the results shown in Table 4. Even at a concentration of 10 $\mu$g/ml, latherin can rapidly become adsorbed and renders Nescofilm partially wettable. Latherin was effective in enhancing surface wettability at concentrations one or more orders of magnitude lower than of the other proteins.

## DISCUSSION

The presence of non-diffusable surface active compounds in horse sweat was demonstrated by a simple capillary-rise method (Fig. 3). Surface activity co-purified by gel filtration and ion-exchange chromatography with a protein for which the name latherin is proposed. Because of their amphipathic nature, proteins generally show some tendency to become adsorbed at interfaces (Cheesman & Davies, 1954; MacRitchie, 1978). However, latherin displayed much greater activity in surface-tension and surface-wetting assays than did any other protein tested (Tables 3 and 4).

Purified latherin appeared homogeneous in the analytical ultracentrifuge and on gel electrophoresis. The electrophoretic behaviour on SDS/polyacrylamide gels before and after reduction (Fig. 4) indicates that the protein has a single polypeptide chain with at least one peptide loop constrained by an intrachain disulphide bond.

The 'protein L' isolated by Eckersall *et al.* (1984) from horse sweat by gel filtration contained the materials presents in peak 3 (Fig. 1), together with the shoulder evident on the trailing edge of the peak, which was not resolved in their separation system. Further contaminating peptides were removed from latherin in the ion-exchange step described above (Fig. 2). Thus 'protein L' contained latherin contaminated with some additional proteins or glycoproteins removed by the more rigorous purification described here. Unlike protein L, latherin contains no detectable hexosamine and is not a glycoprotein.

Latherin has a highly unusual amino acid composition. Almost one residue in four is leucine. The leucine content of latherin (24.9 residues per 100 residues) may be compared with the range of leucine values (1.6–15.5) reported in a sample of 50 non-homologous proteins listed by Reeck (1976). In addition, the total hydrophobic amino acid content of latherin is exceptionally high. The sum of notably hydrophobic residues (leucine, isoleucine, valine, methionine, tryptophan, phenylalanine, tyrosine) is 40.1%, compared with the 25–30% of hydrophobic side chains typical of water-soluble proteins (Tanford, 1980).

The $M_r$ of latherin, calculated from the sedimentation coefficient and Stokes radius, is 24400. This value is consistent with a minimum $M_r$ of 24200 obtained from the amino acid composition. SDS/polyacrylamide-gel electrophoresis indicated a peptide- chain $M_r$ of 20000, whereas gel filtration gave $M_r$ 30000. The discrepancy between these two values for $M_r$ could arise in a number of ways, as both are empirical methods whose validity depends on the assumption that the protein under investigation behaves in the same way as the standards used for calibration. It is possible that the high content of hydrophobic residues in latherin might lead to

**Table 4. Surface adsorption and effect on surface wettability of latherin and other proteins**

Protein was adsorbed on to a hydrophobic surface from solutions of different concentrations. Subsequently the spreading of a drop of buffer on the hydrophobic surface was assessed: (−) contact angle > 90°, (+) contact angle < 90° and (++) spread film of liquid.

| | Protein concn. | | | |
|---|---|---|---|---|
| | 1 mg/ml | 0.1 mg/ml | 0.01 mg/ml | 0.001 mg/ml |
| Ovomucoid | − | − | − | − |
| Bovine serum albumin | + | − | − | − |
| Haemoglobin | ++ | + | − | − |
| Latherin | ++ | ++ | + | − |

anomalous binding of SDS, with consequently enhanced mobility on SDS/polyacrylamide gel electrophoresis leading to an underestimate of $M_r$. An apparently high $M_r$ determined by gel filtration could result from latherin being more asymetrical or hydrated than the globular proteins used for calibration purposes. For a molecule of $M_r$ 24000 and $\bar{v}$ 0.757 ml/g the radius of the equivalent sphere is 1.94 nm. As the observed Stokes radius of latherin was 2.47 nm, the frictional ratio is 1.27. This is a fairly typical value for a globular protein and suggests that latherin is marginally less symmetrical (and/or hydrated) than serum albumin, which has a frictional ratio of 1.30 (Creeth, 1952). As a consequence of its high content of leucine, isoleucine and valine, latherin has a greater partial specific volume than any of the proteins used to calibrate the gel-filtration system. It is possible that the large size of latherin, in relation to $M_r$, contributes to the high value of apparent $M_r$ obtained from gel filtration.

Latherin adsorbs readily to hydrophobic surfaces, rendering them wettable by water (Table 4). This property of the protein may be of functional importance in temperature regulation. Loss of sweat in the form of droplets is clearly less efficient as a means of cooling than is spreading sweat over an animal's hair and skin, with loss of latent heat of evaporation. The capacity of latherin to make hydrophobic surfaces wettable would be compatible with a function for the protein in promoting spreading and evaporation of sweat. Sweating in horses is the major thermoregulatory mechanism (Jenkinson, 1972) and therefore defective secretion of sweat results in the loss of ability to maintain exercise.

In solution latherin behaves as a compactly folded, soluble, globular protein. Adsorption of proteins at interfaces is usually associated with at least partial unfolding (MacRitchie, 1978). There is evidence suggesting that the surface activity of apolipoproteins may arise from the existence of a soluble globular form of protein of unusually low conformational stability, which undergoes partial unfolding at an interface (Scanu *et al.*, 1982). It is possible that the unusual properties of latherin arise in a similar way.

The finding that reduced alkylated latherin has lower surface activity than the native protein suggests that an internal disulphide loop may have some role in the surface activity of the protein.

## REFERENCES


Adamson, A. W. (1982) Physical Chemistry of Surfaces, 4th edn., Wiley, New York

Allen, G. (1981) in Sequencing of Proteins and Peptides; Laboratory Techniques in Biochemistry and Molecular Biology (Work, T. S. & Burdon, R. H., eds.), pp. 26, 31, Elsevier/North-Holland, Amsterdam

Beeley, J. G. (1985) in Glycoprotein and Proteoglycan Techniques: Laboratory Techniques in Biochemistry and Molecular Biology (Burdon, R. H. & van Knipperberg, P. H., eds.), pp. 105–108, Elsevier, Amsterdam

Cheesman, D. F. & Davies, J. T. (1954) Adv. Protein Chem. **9**, 440–501

Creeth, J. M. (1952) Biochem. J. **51**, 10–17

Eckersall, P. D., Beeley, J. G., Snow, D. H. & Thomas, A. (1984) Res. Vet. Sci. **36**, 231–234

Eckersall, P. D., Kerr, M. & Snow, D. H. (1982) Comp. Biochem. Physiol. B **73**, 375–378

Edelhoch, H. (1967) Biochemistry **6**, 1948–1954

Jenkinson, D. M. (1972) Symp. Zool. Soc. London **31**, 345–456

Jenkinson, D. M., Mahon, R. M. & Manson, W. (1974) Br. J. Dermatol. **90**, 175–181

Laemmli, U. K. (1970) Nature (London) **227**, 680–685

Laurent, T. C. & Killander, J. (1964) J. Chromatogr. **14**, 317–330

MacRitchie, F. (1978) Adv. Protein Chem. **32**, 283–326

Neuman, A. W. & Good, R. J. (1979) Surf. Colloid Sci. **11**, 31–91

Reeck, G. (1976) Handb. Biochem. Mol. Biol. 3rd edn. **34**, 504–510

Scanu, A. M., Edelstein, C. & Shen, B. W. (1982) in Lipid–Protein Interactions, vol. 1 (Jost, P. C. & Griffith, O. H., eds.), pp. 259–316, John Wiley and Sons, New York

Siegel, L. M. & Monty, K. J. (1964) Biochim. Biophys. Acta **112**, 346–362

Tanford, C. (1980) The Hydrophobic Effect, 2nd edn., p. 140, Wiley–Interscience, New York

Zamyatnin, A. A. (1972) Prog. Biophys. Mol. Biol. **24**, 109–123


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