ON THE SHAPES OF MOLECULES OF POLY-AMINO ACIDS AND PROTEINS AT INTERFACES

by

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INTRODUCTION

Several attempts have been made to determine the molecular weights of proteins from measurement of the force-area characteristics of these substances when spread in the form of monolayers at either the air-water or the oil-water interface. The method (SCHOFIELD AND RIDEAL[1], BULL[2,3], CHEESMAN[4]) consists essentially in measuring the film pressure F at different values of the surface area, A, available to unit weight of the protein. The product FA when plotted against F often gives an approximately straight line which when extrapolated to zero F gives an intercept $FA = \beta$. This is related, if the extrapolation is justified, to the molecular weight M by the expression $M = 2460/\beta$ when F is measured in dynes cm^{-1} and A in square metres per mg.

Apart from the difficulty in exact extrapolation additional assumptions are tacitly involved. These are that the molecules in the film must be completely rigid and that they must not bear a strong electrical charge.

Whilst the molecular weights of serum albumin (70,000) and human methaemoglobin (66,000) are normal at the air-water surface, apparent values of 4,400 and 8,200 respectively have been obtained at the oil-water interface by CHEESMAN[4]. Although he postulated dissociation in the interface to explain these values, his alternative theory, that there is a different molecular orientation at the two interfaces, will be shown to be more probable. The peculiar orientation at the oil-water interface must involve extensive unfolding of the molecular chains (surface denaturation); at the air-water surface this occurs to a very much lesser degree. This will account for the apparently low molecular weights.

With the object of throwing more light on the behaviour of such monolayers at these interfaces we have examined a number of synthetic poly-amino acids of known constitution. Interfacial dissociation of these is practically ruled out.

The different lines of evidence for interfacial unfolding may be summarised as follows:

1. The surface viscosities of films of proteins are very much higher at the oil-water interface than at the air-water surface. This supports the view that in the former case the polypeptide chains are more extended and flexible, tending to become intertwined.

2. The data of CHEESMAN are entirely consistent with the concept of unfolding.

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According to the equations of SINGER[5] this leads to non-linear FA–F plots even at low pressures.

3. Unless the data are interpreted in terms of changes in shape of the molecules, the poly-amino acids would have molecular weights in the interface less than the minimum values indicated by end-group analysis.

4. The surface potentials of films of proteins and poly-amino acids at the interfaces between air and water and between oil and water are different, and are interpreted below in terms of unfolding at the oil-water interface.

5. The general composition of proteins is such that cohesion between hydrocarbon groups within each molecule will normally be expected to be very powerful. When oil is present to eliminate this cohesion a looser structure will inevitably result.

It is concluded from the data summarised in Table I that the protein molecules are unfolded at the oil-water interface into long chains of considerable flexibility (Fig. 1a). The molecular weight of these is still not known conclusively although to explain the experimental data it is not essential to assume any dissociation. At the air-water interface the molecules will be held together by the attraction of the hydrocarbon chains and rings to give a form shown diagrammatically in Fig. 1c. In bulk solution the protein molecule is more or less spherical with the hydrocarbon chains directed towards the centre; the different possible superficial configurations represent different degrees of a particular type of denaturation. Some calculations along these lines are given below.

TABLE I

TYPICAL DATA FOR PROTEINS

Quantity Measured	*Value at A/W surface*	*Value at O/W interface*
Flexibility, ω	0.015	0.12
Surface viscosity at $F = 4$ dyne cm^{-1}	0.01 surface poises (JOLY[11])	0.1 surface poises (CUMPER AND ALEXANDER[12])
Surface dipole moment, μ, per amino acid residue (Hb. at pH 6.8 at 1 m^2. mg^{-1})	124 m.D.	78 m.D.
Entropy of surface denaturation per amino acid residue		
(a) in isolated protein molecules	+ 0.03 e.u.	+ 0.22 e.u.
(b) in film compressed to 1 m^2 mg^{-1}	— 0.01 e.u.	0.00 e.u.

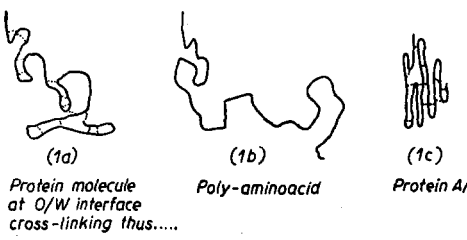

Fig. 1. (a) The configuration of a protein chain at the oil-water interface. There is considerable flexibility, although remaining cross linkages, (shown by ....), hinder the thermal intramolecular movements. (b) Poly-amino acid chain at the oil-water interface. Here the flexibility is practically complete, and the chain can take up any shape under the action of thermal agitation. In both cases the oil has penetrated between the non-polar side-chains and eliminated their cohesion. (c) A protein molecule at the air-water interface. The molecule is folded on itself in two dimensions owing to the action of powerful cohesive forces between the non-polar side chains.

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EXPERIMENTAL

Apparatus

In the use of a hanging plate to measure the lowering of the interfacial tension, F, the technique was similar to that of CHEESMAN[5,6]. There were, however, two points of departure from the established practice. Firstly, the plate was made of very thin mica, with a very small buoyancy effect. The plate was rubbed with a fine emery paper and then rendered hydrophobic with carbon-black[6] deposited from a flame of burning paraffin wax. Such a plate was found satisfactory even if the oil was lighter than water if the plate was always restored to its initial position during the measurements. This constituted the second point of departure. The plate, instead of hanging from the arm of a balance, was attached to the end of a ten cm arm joined at its other end to a fine torsion wire. A calibrated scale attached to one end of this torsion wire gave the force required to restore the plate to its initial position. A small mirror attached to the arm close to where it was secured to the wire permitted accurate measurements, an overall error of not more than 0.02 dynes cm^{-1} being estimated.

Procedure

The general method of carrying out the measurements was closely similar to that of CHEESMAN[4]. Since, however, the experiments lasted only about 10 minutes, no effort was made to thermostat the apparatus, the temperature of which was $21 \pm 1^{\circ}$ C.

Materials and methods

Reagents were of AR quality. Liquids were purified by distillation in an all-glass apparatus. The copolymer glutamic acid-lysine was spread from a solution in water containing 50% *iso*-propyl alcohol, and the poly-DL-alanine from solution in water containing 50% ethanol. The copolymer γ-methyl glutamic acid-phenylalanine and the poly-DL-leucine and poly-DL-phenylalanine were dissolved first in pure dichloracetic acid. After solution was complete about 20% *iso*propyl alcohol could be added without causing any precipitation. Such a solution is apparently of general application for spreading monomolecular films of the non-polar poly-DL-amino acids. The spreading operation should be performed very slowly, especially if the film is finally being spread against a considerable surface pressure. The solvent itself has but a transitory effect on the surface pressure and on the surface potential. No evidence of catalytic depolymerization was found, although as a precaution against this all experiments were conducted shortly after the solution was prepared.

The copolymer of glutamic acid and lysine was kindly provided by Dr. H. TANI. Its preparation is described by AKABORI, TANI AND NOGUCHI[7]. The sample used had a Mol. wt. of 5,700 as deduced from osmotic pressure measurements[7]. The poly-DL-alanine was prepared by Dr. C. E. DALGLIESH, and consisted of about 50 residues[8]. The remaining poly-amino acids were provided by Courtaulds, Ltd. The horse haemoglobin was given by Dr. D. F. CHEESMAN.

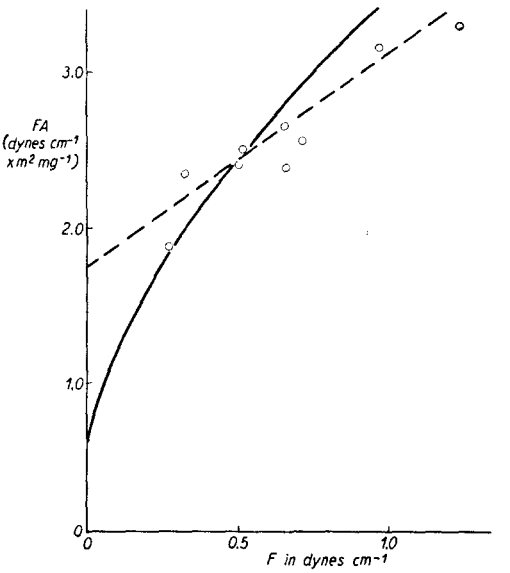

Fig. 2. FA as a function of F for poly-DL-alanine, 50-mer, at the interface between petrol-ether and $N/100$ HCl. The units of A and F are $m^2 mg^{-1}$ and dynes cm^{-1} respectively. The broken line is a linear extrapolation through the experimental points, which leads to a value of $2460/1.75 = 1400$ for the apparent molecular weight. The full line represents equation (3) in which $z = 3.33$ and $x = 50$, corresponding to the analytical M.Wt. of 3568.

RESULTS

The variation of the product FA with F is shown for polyalanine in Fig. 2. The points are the experimental values at the benzene-water interface while the curve is the theoretical equation of SINGER (see below) fitted to the points. The dashed line shows the straight line drawn through the points which, if extrapolated to the FA axis, leads to an impossibly low value for the molecular weight of the polymer.

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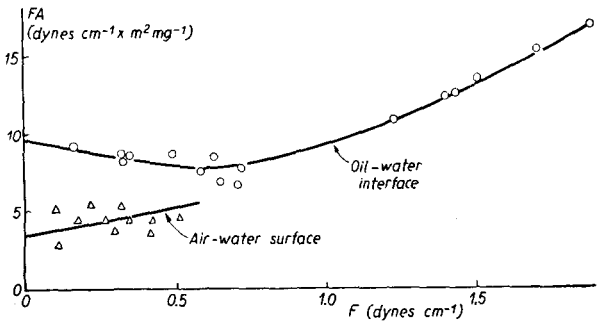

Fig. 3. *FA* vs. *F* plots for the 1:1 copolymer of γ-methylglutamic acid and phenylalanine spread at the interface between air and *M*/10 phosphate buffer at pH 6.8 (–Δ–). The units of *F* and *A* are the same as in Fig. 2. The linear extrapolation gives an apparent M.Wt. of 2460/3.3 = 750, compared with the value of 1450 from end group analysis (ROBINSON[23]). The plot for the benzene-aqueous interface is also shown, (–o–). The minimum in the curve is due to charge effects (the carboxyl groups will be ionized at this pH). The apparent molecular weight is now only 260.

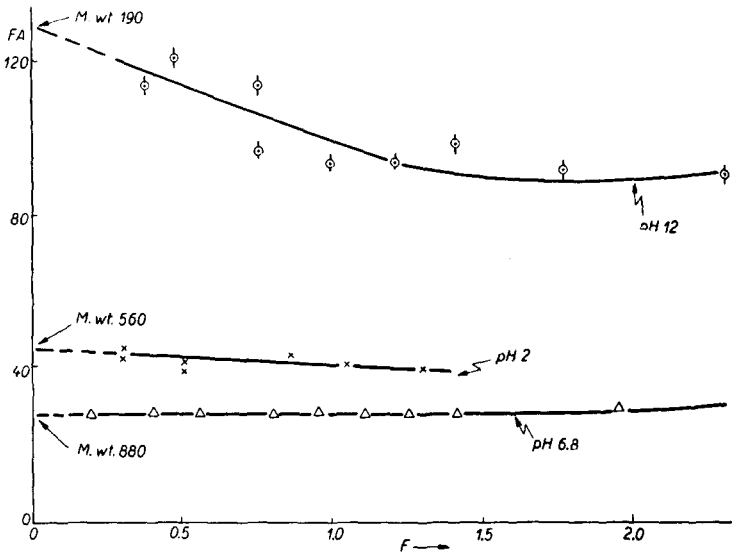

Fig. 4. *FA* vs. *F* curves between petrol-ether and water for a 1:1 copolymer of glutamic acid and lysine. The apparent molecular weight depends on the electrical charge, values being 880 (neutral film, *M*/50 phosphate pH 6.8); 560 (positive film, pH 2); and 190 (negative film, pH 12). Since the –CO–NH– bonds cannot be broken under these conditions, linear extrapolation of these parts of the curves to obtain molecular weights is clearly unjustified. Units and calculations as in Fig. 2; molecular weight of polymer 5700.

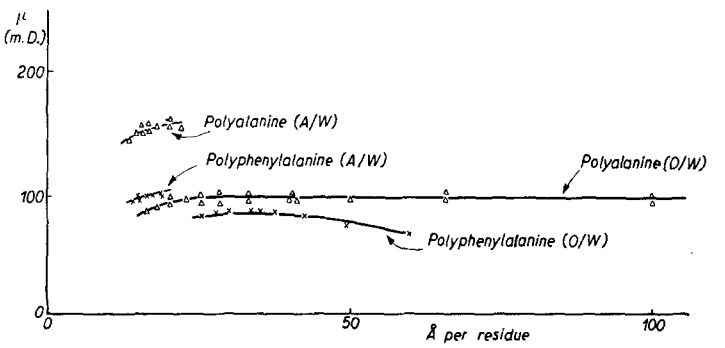

Fig. 5. Surface dipole moment, μ, per residue in films of poly-DL-phenylalanine (–x–) and of poly-DL-alanine (–Δ–) at the interfaces between air and water and petrol-ether and water (pH 6.8, *M*/50 phosphate buffer).

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Fig. 3 shows the difference in the FA vs. F curves of γ-methylglutamic acid—phenylalanine copolymer at the air-water and oil-water interfaces, while Fig. 4 shows more explicitly than Fig. 3 the effect of electrical charge on the FA vs. F curves (glutamic acid-lysine copolymer). Surface dipole moments at the two interfaces for polyalanine and polyphenylalanine are shown in Fig. 5. They are calculated from interfacial potentials[9] using the Helmholtz formula, $\Delta V = 4\pi n\mu$. Here n is the number of aminoacid residues per cm^2 of surface. Similar quantities are shown for haemoglobin at the isoelectric point in Fig. 6.

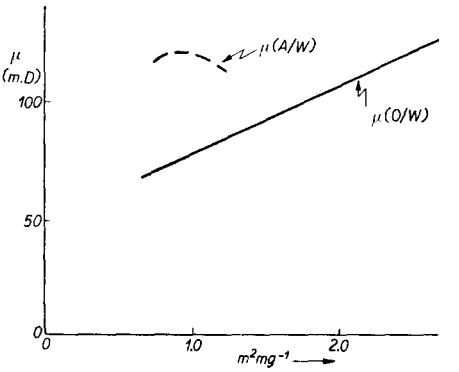

Fig. 6. Surface dipole moment curves at air-water and petrol-ether-water interfaces for horse haemoglobin (pH 6.8, $M/50$ phosphate). Values are averages per amino acid residue.

DISCUSSION

Surface viscosity and unfolding of molecules

That the viscosity of a protein solution increases on denaturation is well known[10]. This, combined with the fact that the surface viscosities of proteins at the air-water interface are very low[11], supports the view that here the peptide "backbones" are coiled rigidly into a relatively compact structure of the type shown in Fig. 1c.

At the oil-water interface the cohesion within the coil is removed. The film viscosity is *increased* by as much as 10 fold (*cf.* results of CUMPER AND ALEXANDER[12]) indicating that the molecules have become relatively very long and thin. The data are summarised in Table I. This shows that the presence of the oil causes the chains to unfold. Had dissociation into "sub-molecules" occurred, the interfacial viscosity would have *decreased* considerably.

Unfolding and the surface equation of state

Force-area plots of macromolecules may be used to deduce quantitatively the degree of unfolding of molecules in an interface. Indeed, this procedure furnishes an extremely sensitive method of determining the shape of large molecules in a surface, provided only that they are neither strongly repellent nor strongly attractive towards each other.

For small, compact molecules the equation of state is $F\underline{A} = kT/x$, where $\underline{A}$ is the area occupied by a small molecule or by each repeating unit of a polymer and x is the number of these units in the whole molecule. This "ideal" equation is often obeyed at relatively low pressures. However, at higher pressures serious deviations are found. If a correction is made to take account of $\underline{A}_o$, the actual area occupied by each unit in the interface, the "ideal" equation becomes $F(\underline{A} - \underline{A}_o) = kT/x$. This is the equation which BULL[3] has successfully applied to films of proteins at the *air-water* surface. Both theoretical equations predict linear plots of FA against F, from the intercept of which on the FA axis x and hence the molecular weight may be easily determined.

A different equation of state may be derived assuming the existence in the surface of definite "sites", on each of which is held either a segment of the molecule constituting the film or a water molecule. The simplest form of such an equation of state is:

$$F = \frac{-kT}{x\underline{A}_o} \ln (1 - \underline{A}_o/\underline{A}) \qquad (1)$$

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which, if A is much greater than A_0 becomes

$$F\,(\underline{A} - \underline{A}_0/2) = kT/x \qquad (2)$$

and which in the limit when $\underline{A}/\underline{A}_0$ tends to infinity ($F \to 0$) leads to the simple form $F\underline{A} = kT/x$. Equation (2) also implies a rectilinear plot of FA against F.

All the above equations apply to rigid molecules only. The treatment involving the "sites" has, however, an additional advantage in that it may be extended to flexible molecules. For this SINGER[5] made the necessary calculations using the basic ideas of the Florey-Huggins treatment of high polymers in bulk solution to calculate the relatively large steric activity coefficients. If the molecules constituting the film are completely rigid, each unit in the chain has exactly two positions, one on each side of it, on which neighbouring units, *e.g.* the amino acid residues, can be fitted as shown in Fig. 7a. This is expressed quantitatively in terms of a coordination number, z, which in this case is 2. For such a rigid chain equation (1) fits the experimental data.

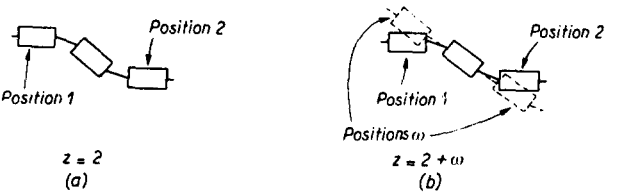

Fig. 7. (a) Representation of the arrangements of the segments of a rigid molecule of a polymer. Each segment on the surface has exactly two positions in which its two neighbours in the chain must fit. Thus, when the co-ordination number, z, is 2 there is no flexibility in the polymer chain. (b) If there are more than two possible positions around each segment for its neighbours, some bending of the chain in the surface becomes possible. The flexibility, ω, is the amount by which z, the co-ordination number, exceeds two.

If the coordination number z is greater than 2 by an amount ω, so that $z = 2 + \omega$, a certain amount of flexibility measured by ω characterises the configuration of the chains in the surface. This is illustrated in Fig. 7b. Complete flexibility in which every unit in the chain could bend at right-angles to its neighbour should correspond approximately to $z = 4$ or $\omega = 2$. In practice, however, especially if the molecule be large, the chains when at high areas will be able to assume a wide variety of shapes for values of ω as low as 0.1.

The relation between F, A and z deduced by SINGER[5] is given to a close approximation by:

$$F = (kT/\underline{A}_0)\left(\frac{(x-1)}{(x)}\left(\frac{z}{2}\right)\ln\,(1 - 2A_0/zA) - \ln\,(1 - A_0/A)\right) \qquad (3)$$

If $z = 2$ this reduces to the form (1) above. As shown in Fig. 8, F increases very markedly with z, wherein lies the sensitivity of this method of determining z and ω from the force exerted by the film. In just the same way the osmotic pressure of solutions of high polymers is increased by flexibility of the molecules.

In the limit when A/A_0 becomes very large the equation (3) tends to the form $F = kT/x\underline{A}$, irrespective of the value of z, although unless z be very close to 2, *i.e.* the flexibility ω is extremely small, the plot of FA against F will not be linear. An example of this may be seen in Fig. 9, where the upper line represents the plot of (3) for $z = 2.12$, ($\omega = 0.12$).

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SINGER's equation has been fitted to the experimental data for the poly-amino acids spread at the oil-water interface, as shown in Fig. 2. For both poly-DL-leucine ($x = 200$) and for poly-DL-alanine the value of ω is quite high, 1.33 in each case. Here z approaches its limiting value of 4, and the poly-amino acid chains undoubtedly have very great freedom of bending and twisting, as in Fig. 1b. The interchain hydrogen bonds are evidently broken. For the polyalanine x was 50, *i.e.* Mol. wt. = 3568, as deduced by end-group analysis[8]. It was not possible to obtain this from the extrapolated value of FA at zero F, which is scarcely surprising in view of the marked deviations from linearity expected from (3) when z is so far from 2.

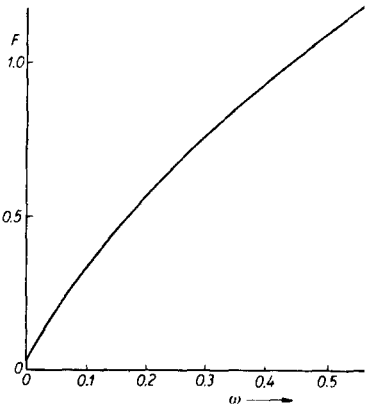

Fig. 8. The increase in F with ω is very rapid according to equation (3). Data are for human methaemoglobin at 2 m^2 mg^{-1} at the iso-electric point. The force exerted by the film permits an accurate estimation of ω, the flexibility.

At the air-water interface protein films obey (3) well with a coordination number of 2.015[5]. The very small flexibility implied in the low value of ω of 0.015 suggests that the approximate linearity of the FA vs. F relation of equation (1) above may well hold here, especially in view of the fact that (3) tends to (1) when $z = 2$. This is indeed the case, as shown in Fig. 9 from the data of SINGER[5] and of BULL[2]. It seems valid, therefore, at the *air-water* surface to extrapolate the FA vs. F relation to zero F to obtain the molecular weights of proteins. KALOUSEK[13] has reported an apparatus suitable for measuring the necessary low pressures.

For proteins at the *oil-water* interface CHEESMAN has recorded that even at the highest areas the film pressure F is still considerably greater than at the air-water interface. If now, instead of assuming as he did that x is reduced at the oil-water interface, with the implicit assumption in the linear extrapolation that z is very close to 2, we now substitute for x the normal value of about 530 for the number of amino acid residues in human methaemoglobin and apply (3) to determine z, we find a value of 2.12 for the latter. The flexibility, ω, is thus 0.12, about 10 times the value for proteins at the air-water interface. That the protein has become relatively flexible, having unfolded from the shape shown in Fig. 1c to something of the form shown in Fig. 1a, is supported by the

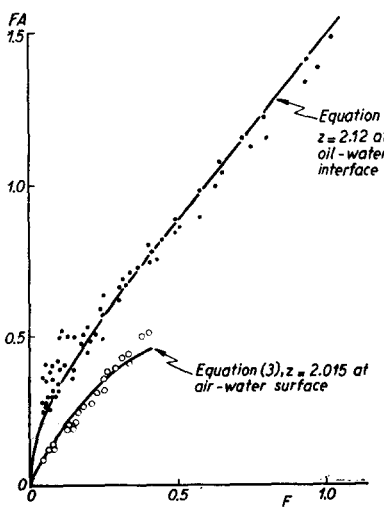

Fig. 9. Experimental points (shown ●) of CHEESMAN[4] for FA vs. F for films of human methaemoglobin at the interface between benzene and 0.001 M phosphate buffer at pH 6.8. To these equation (3), represented by the full line, has been fitted. The molecular weight is assumed normal while the flexibility is 0.12. Extrapolation to zero F would require data at much lower interfacial pressures than hitherto attainable experimentally. Also shown (o) is the product FA vs. F for egg albumin at the air-water interface[2]. The full line is equation (3) with $\omega = 0.015$[5]. When ω is so small linear extrapolation from the experimental data is possible both theoretically and practically.

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other types of measurements shown in Table I. This can explain simply the apparently low molecular weights reported[4] from the linear extrapolation of the FA vs. F curves, since as shown in Fig. 9 the plot may be expected to show a very marked curvature at low pressures. Experimentally, one would have to work at exceedingly small pressures, probably of the order of a few millidynes cm^{-1}, before being able to determine by extrapolation these molecular weights at the oil-water interface.

CHEESMAN, whose data are certainly the best available was unable to obtain results in this region. It seems, indeed, unlikely that more accurate results are possible, on account of the difficulties of removing all traces of contamination from an oil-water interface. Instead, the additional evidence is provided by measurements of interfacial viscosity and interfacial potential as well as by the high values of ω for the poly-amino acids. The latter are clearly random chains under these circumstances, and it is concluded that the observed changes for both poly-amino acids and proteins can be most simply explained in terms of molecular flexibility.

Confirmation of interfacial configurations from potential measurements

It has already been reported[9] that the interfacial potentials, ΔV, are much higher at the air-water than at the oil-water interface for poly-alanine and poly-leucine. This may also be expressed in terms of the surface dipole moments, μ, related to ΔV by the expression $\Delta V = 4\pi n\mu$, where n is the number of amino acid residues per square cm of interface and μ is expressed in millidebyes per residue. At pH 6.8, for example, the moment for poly-leucine is 145 m.D. at the air-water interface and 100 m.D. at the oil-water interface. The oil used in the interfacial potential measurements was always petrol-ether. Poly-DL-phenylalanine and poly-DL-alanine had the moments shown in Fig. 5. The moments of these polymers containing only hydrocarbon side-chains depend primarily on the orientation of the C = O groups. The more nearly vertical these become, in the sense of the oxygen being oriented towards the water, the closer will be the potential approach a maximum value of 360 m.D.[14]. On the basis of these potential results, combined with the established data on the areas to which the films may be compressed, at the two interfaces, the arrangements of the chains of the poly-amino acids (including polyalanine, *cf.* CUMPER AND ALEXANDER[12]) are probably those shown in Fig. 10.

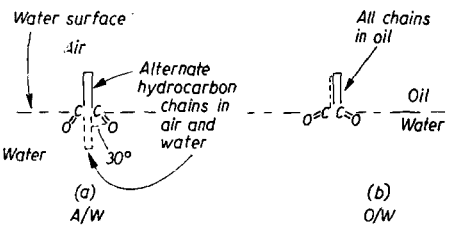

Fig. 10. Orientations of the poly-amino acids as deduced from potential measurements. At the air-water interface, (a), the side-chains alternate above and below the water surface. The C = O groups point outwards from the double chain at an angle of about 30° with the vertical, as suggested by the relatively high μ values. To stabilise this structure, the chains below the surface must have close neighbours with which Van der Waals interaction is possible. This prevents free bending of the molecules in the surface. At the oil-water interface, (b), oil penetrates between the side-chains, and they swing round till all are immersed in the oil phase. The C = O groups are now at a greater angle, 60°, and μ is reduced. Adhesion to neighbouring chains is unnecessary to stabilise this structure, and the chains can flex easily.

It is important to note that at the air-water surface the area per residue (18 A^2) in the compressed film of poly-leucine is only about one half of the area of each side chain, (30 A^2). To account for this it is necessary to assume that even a non-polar hydrocarbon chain such as $-CH_2-CH<^{CH_3}_{CH_3}$ in the leucine residues can be pushed below

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the water surface. This is evidently possible on account of the Van der Waals forces between the chains both above and below the water surface. A similar effect is reported (ALEXANDER AND SCHULMAN[15]) for long-chain esters, where again hydrocarbon chains can be pushed below the water surface. That the film of the poly-amino acid should be stable (collapse pressure about 16 dynes cm^{-1}) under these circumstances is perhaps surprising. Evidently the C = O groups, all oriented towards the water, stabilise the folded structure shown in Fig. 10a. In it the chains are held togetherr igidly by Van der Waals forces, and a scale model shows very little flexibility.

Not so the model of the oil-water system; the chain can be freely bent so that each residue is almost at right-angles to its neighbour, in qualitative accord with the high value for the flexibility, ω, of 1.33. This is shown in Fig. 10b. The cohesion within the film at the air-water surface is very high, and application of (3) to determine z and ω would be unjustified.

That replacement of the air by oil should have so striking an effect on the configuration of the polymers in the interface is comprehensible if it is realised that the Van der Waals forces of cohesion between neighbouring hydrocarbon side-chains stabilise the air-water form. Once oil is present, it will penetrate into this mass of cohering side-chains, reducing almost to zero the cohesion between neighbours. The stabilising forces being thus removed by the oil, there is nothing to prevent all the side-chains lying immersed in the oil, as shown in Fig. 10b.

In protein films the orientations are essentially similar to those in the poly-amino acid films, since the dipole moments are close to those for poly-amino acid films (Fig. 6). There will doubtless be some tendency, however, for the more polar side-chains to be oriented towards the air, though this, by analogy with the poly-amino acids, is not essential for film stability. The position of the polar side-chains will be discussed in detail elsewhere. Further, in "gaseous" protein films each molecule will be separated from its neighbours by a fringe of polar side-chains lying flat on the water surface. These will reduce almost to zero the cohesion between different protein molecules, although at the air-water surface the Van der Waals forces of attraction between different parts of the *same* molecule are high, and the flexibility ω is only 0.015.

Effect of an electrical charge on the molecule

If any molecule contains ionised groups, there will be repulsion between it and its neighbours[16]. A flexible chain will also become more extended if there are several charged groups attached to it. The magnitude of these effects cannot be calculated exactly for larger molecules, yet it is certain that both will increase the values of FA, especially as the pressure falls. At extremely low pressures, however, there will be a decrease in FA, which will tend to the value for an unionised film as F tends to zero. The latter condition seems never to be realised experimentally with molecules as large as poly-amino acids or proteins, so that what is observed for these at the oil-water interface (when cohesion is minimised) is that FA increases as F is reduced and that extrapolation of such data to zero F gives a false value of the molecular weight.

As an example, the results in Fig. 4, suggest that the apparent molecular weight of the glutamic acid-lysine copolymer at the oil-water interface is considerably lowered on acid or alkali, the values being respectively 560 and 190. From osmotic pressure measurements the molecular weight was deduced to be 5,700, showing that the extrapolation of the curve to zero F is invalid. The possibility of splitting the peptide bonds

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under these conditions must be ruled out. Even at the iso-electric point the apparent molecular weight is as low as 880, presumably because the unfolded chains are flexible. There is no reason to think that in all cases the correct molecular weight could not be obtained if the extrapolation could be performed from sufficiently low pressures.

It seems reasonable to attribute the minimum in the plot of FA vs. F which CHEESMAN[4] found for serum albumin at pH 6.8 (I.P. at pH 4.8) to a charge effect. The negative charge on the protein would be expected by analogy with these results (Figs. 3 and 4) to lead to an increase in FA at the lowest pressures attainable experimentally. Linear extrapolation cannot give the true molecular weight in such a complex case. A similar effect for the osmotic pressures of bulk solutions of polymers is recently reported[17].

Interfacial denaturation: associated entropy changes

The breakdown of the forces responsible for rigidly maintaining a particular structure in the protein molecule is generally referred to as denaturation. On this basis, as has already been stated on quantitative grounds by DANIELLI[18] and ALEXANDER[19], spreading at the oil-water interface is a form of denaturation. This suggests that many, but not all, of the links holding the native protein in its characteristic configuration are of the Van der Waals type, operating unspecifically between the hydrocarbon chains of residues such as leucine and phenylalanine. That the flexibility of the proteins at the oil-water interface (0.12) is so much less than that of the poly-amino acids (1.33) indicates that the protein retains some restrictive cross-links, possibly of a polar character. DANIELLI reached the same conclusion by different means.

We may measure the degree of denaturation at different interfaces by means of the *entropy of denaturation.* This quantity, it must be stressed, is related to the equilibrium between the native and the denatured protein or poly-amino acid; it bears no direct relation to the rate of denaturation. It measures the degree of randomness achieved by the denatured protein molecule relative to that of the native protein.

When A is very large and the chains are virtually independent, the entropy S_s of each in the surface, due to its constant bending and coiling under the influence of the thermal motion of the solvent molecules is:

$$S_s = (x - 2)\, R \ln (z - 1) \tag{4}$$

which may be deduced as a limit of an expression of SINGER[5]. Expressed as the entropy s_s per amino acid residue this becomes:

$$s_s = (1 - 2/x)\, R \ln (z - 1) \tag{5}$$

For a rigid molecule ($z = 2$), this becomes zero as we should expect, since no randomness due to bending is now possible. For human methaemoglobin at pH 6.8 at the oil-water interface $z = 2.12$ and hence $s_s = 0.22$ e.u. These are larger (contrast ALEXANDER[19]) than the values (for egg albumin) at the air-water interface: $z = 2.015$; $s_s = 0.03$ e.u. (Here e.u. refers to entropy units). Since there is evidence that these values depend but slightly on the particular protein studied, we can say quite generally that while the entropies per amino acid residue of proteins are much greater at the oil-water than at the air-water interface, they are still well below the figures of 1.6 e.u. for each residue of the poly-amino acids polyleucine and polyalanine spread at the oil-water interface. These entropy figures are equal to the entropies of surface denaturation for the isolated

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protein chains if we assume that the rigidity of the native protein is complete, *i.e.* that $z = 2$ and hence $s_s = 0$. This last assumption seems reasonable in view of the suggestion of PAULING AND COREY[20] that a definite helix may persist in globular proteins.

If now the very dilute film be compressed, the chains begin to touch one another. This reduces somewhat their ability to bend and at the same time increases the surface pressure. Since the latter would still increase slightly, even if the molecules were rigid (because of the increased collision frequency) allowance must be made for this. It is equal to the corresponding change in the "ideal" film in which $z = 2$.

The decrease in entropy Δs_s as the film is compressed may be obtained from the expression:

$$s_s = (1/xT) \int_{A=\infty}^{A} F \, dA \;(z = z) \; - \; (1/xT) \int_{A=\infty}^{A} F \, dA \;(z = 2)$$

and may be simply computed by a graphical method from the force-area curves. The results are shown in Table II.

TABLE II

System	s_S *for independent molecules (Entropy of surface denaturation per residue)*	Δs_S *(Change in* s_S *as film is compressed to* 1 m^2 mg^{-1}	$s_S + \Delta s_S$ *(Total entropy of surface denaturation* 1 m^2 mg^{-1}
Poly-amino acids (poly-DL-leucine and poly-DL-alanine) at O/W interface	+ 1.62 e.u.	— 0.85 e.u.	+ 0.77 e.u.
Protein at O/W interface (Human methaemoglobin) (Calculated from data of CHEESMAN[4])	+ 0.22 e.u.	— 0.22 e.u.	0.00 e.u.
Protein at A/W interface (egg albumin) (Calculated from data of BULL[2])	+ 0.03 e.u.	— 0.04 e.u.	— 0.01 e.u.
Rigid molecule ($z = 2$) at any interface	0.00 e.u.	0.00 e.u. (by definition)	0.00 e.u.
Trypsin (thermal denaturation) (STEARN AND EYRING[22])	s for thermal denaturation = + 0.76 e.u.	—	—

It is interesting to compare s_s with the figure of 0.76 e.u. obtained from the results of ANSON AND MIRSKY[21] by STEARN AND EYRING[22]. This refers to the thermal denaturation of each residue of trypsin, and is in excess of the maximum entropies in the surface denaturation of proteins. It seems that thermal denaturation causes the breaking of some of the linkages unaffected by interfaces, although even here the opening of the molecular structure is less complete than for the poly-amino acids.

Significance of the shape of protein molecules

Biologically, the interest of the results described here is that if proteins in a living cell are adsorbed at a lipid water interface, or if they are combined to form lipoproteins,

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they will be partially extended into long chains. Not only is their high specificity now explicable without long-range forces; not only is the problem of understanding protein synthesis simplified; but the long, thread-like molecules, because of their flexibility, may interact easily with other protein molecules, changing specifically the activities of both the lipid surfaces and those of neighbouring enzymes.

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SUMMARY

1. Unimolecular films of poly-amino acids at the oil-water interface behave as though the molecules were long, freely flexible chains, in which hydrogen bonds between chains are evidently not maintained.

2. The existing data for proteins at the *oil-water* surface can be interpreted in the same way. This is supported by the known values of surface viscosity and the recently measured interfacial potentials. At the *air-water* interface, however, a protein molecule is coiled up due to the action of Van der Waals forces between the non-polar side-chains.

3. Protein molecules at the air-water and oil-water interface are in different degrees of surface denaturation. The corresponding entropies have been calculated.

RÉSUMÉ

1. Des films monomoléculaires de polyaminoacides à l'interface huile-eau se comportent comme si les molécules étaient de longues chaines flexibles, dans lesquelles les liaisons hydrogène entre les chaines ont évidemment disparu.

2. Les données existantes pour des protéines à l'interface *huile-eau* peuvent s'interpréter de la même façon, ainsi que le montrent les valeurs de la viscosité de surface et les potentiels interfaciaux récemment mesurés. A l'interface *air-eau*, cependant, une molécule protéique est repliée par l'action des forces de Van der Waals s'exeréant entre les chaînes latérales non-polaires.

3. Les molécules protéiques aux interfaces air-eau et huile-eau sont dénaturées à des degrés différents. Les entropies correspondantes ont été calculées.

ZUSAMMENFASSUNG

1. Einmolekulare Filme von Polyaminosäuren verhalten sich an der Zwischenfläche Öl/Wasser als ob die Moleküle lange, frei biegsame Ketten wären, bei denen die Wasserstoffbindungen zwischen den Ketten offensichtlich nicht aufrecht erhalten bleiben.

2. Die bestehenden Daten für Proteine an der Öl/Wasser Oberfläche können in der selben Weise interpretiert werden. Dies wird durch die bekannten Werte der Oberflächenviskosität und die kürzlich gemessenen Zwischenflächenpotentiale unterstützt. An der Zwischenfläche Luft/Wasser jedoch wird ein Proteinmolekül aufgerollt, was der Wirkung der Van der Waals'schen Kräfte zwischen den nicht polaren Seitenketten zuzuschreiben ist.

3. Proteinmoleküle an den Zwischenflächen Luft/Wasser und Öl/Wasser befinden sich in verschiedenen Stadien der Oberflächen-denaturierung. Die dazu gehörigen Entropien wurden berechnet.

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