STRUCTURE AND SURFACE PROPERTIES OF PROTEIN MONOLAYERS

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ABSTRACT

The role played by monomolecular films of proteins and other biological materials in supporting the metabolic functions of a living cell has become more fully recognized in recent years. Thus the relevance of a study of films of these materials in the laboratory in an effort to determine their method of formation, nature, and mode of action within the cell becomes obvious. As a brief introduction to this area of research, a general review relating to the surface properties of proteins is presented. This includes discussion of the structure of protein molecules, surface denaturation and associated entropy changes, the formation and physical characteristics of protein films, the mechanisms associated with film compression, and the intramolecular and intermolecular forces of attraction and repulsion existing within the films.

PROBLEM STATUS

This is a final report on one phase of the problem; work on other phases is continuing.

AUTHORIZATION

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STRUCTURE AND SURFACE PROPERTIES OF PROTEIN MONOLAYERS

INTRODUCTION

The role played by monomolecular films of proteins, lipids, and other biological materials at the many interfaces existing in a living system, has, in recent years, become more fully appreciated. Today it is generally recognized that these films are integrally involved with many of the vital life processes of the living cell. For this reason it becomes of considerable importance that information be obtained concerning the behavior of these biological materials as they are thus extended. Unfortunately an idealized study of these materials as they exist in their truly native state in an undisturbed living cell is very difficult if not impossible to achieve. The alternative is to prepare films of these materials in an artificial environment and to attempt a careful extrapolation of the information obtained.

Accordingly this Laboratory has been actively engaged in an investigation of the chemical and physical characteristics of monomolecular films of certain biologically active proteins and the effect on their monolayer formation induced by the complexing of these proteins with an inhibitor which is also proteinaceous in nature. Of specific interest are the interacting protein systems catalase-ovomucoid described by Abrignani and Mutolo (1), trypsin-ovomucoid described by Lineweaver and Murray (2), and chymotrypsin-ovomucoid described by Weil and Timasheff (3).

To date this Laboratory has completed studies on catalase (4), ovomucoid (5), and the catalase-ovomucoid complex (6). Work on other phases of the investigation is still continuing. The purpose of this report is to present to the reader not familiar with this area of research, a basis understanding of the nature of proteinaceous materials and the mechanisms involved in the unfolding of these materials into monomolecular films.

SURFACE-SPREAD PROTEINS AND THE LIVING CELL

The physical properties of protein films formed at an air-aqueous interface were first studied by Devaux (7), who investigated the nature of “albumin” films on pure water. Since that time this area of physical biochemistry has been expanded and refined to a considerable extent, until today it offers one of the more promising routes by which a detailed study may be made concerning intracellular behavior of biologically active materials.

The importance of surface phenomena in biology has long been recognized (8). This surface phenomena resides within the cell, the basic structural unit of a living organism. Each cell contains, within its boundaries, many inclusion bodies such as the mitochondria, microsomes, and the nucleus with its own inclusions. These inclusion bodies are separate phases distinct from the aqueous cytoplasm of the cell and separated from it by an interfacial region. In addition, the boundary of the cell constitutes another interfacial region between intracellular protoplasm and extracellular fluid. Because there are millions of cells comprising the animal body there are many such interfacial regions. A large portion of the cellular proteins are found to be located at these interfaces in the form of lipoid-protein complexes (9) in which the protein is present in a partially or completely unfolded state. Some of these proteins are concerned with cell structure (10), while others, chiefly
enzymes, are more localized within the cell (mitochondria), and function to expedite anabolism and catabolism of major cell components. These are in addition to the proteins, fats, nucleotides, and carbohydrates. The metabolism of fats (and steroids) may be considered as a good example of the occurrence of interfacial reactions within the cell. Fats are, in general, so insoluble in aqueous media that the final stages of synthesis and initial stages of utilization must occur either within a lipoid phase or at a lipoid/water interface. Since the enzymes catalyzing these reactions are not lipoid-soluble, it must be concluded that the reactions must be limited to an interfacial region (11).

If most intracellular proteins exist as ill-defined films, the relevance of surface chemistry techniques to their study is obvious. Of particular importance in such an investigation would be the study of the intracellular enzymes. The catalytic activity of an enzyme toward its natural substrate, as described above, appears to be a surface phenomenon. Even in bulk reactions the size of the enzyme molecule is such that the reaction that is catalyzed must occur at its surface (11). It would be of great interest to study these catalytic systems with the biocatalyst extended at a suitable interface; a considerable amount of work has already been done in this area (12-15).

The general mechanism by which proteins unfold at an interface does not appear to be affected by any inherent biological activity that a protein may possess. In general, proteins capable of film formation exhibit similar surface characteristics irrespective of the presence or absence of such activity. This is not surprising because all proteins are composed of a limited assortment of rather similar building blocks (exclusive of enzyme prosthetic groups such as the hemin of catalase or the sugar moiety of ovomucoid). There are, of course, variations in structure from protein to protein; however, these variations are not of such a drastic nature that they impart any great change in magnitude to the parameters associated with film studies.

General information may be obtained from the study of the surface characteristics of a given protein that would be applicable to other proteins as well, and such information has, indeed, been obtained over the years. A great many proteinaceous materials have been subjected to a study of their film characteristics since the first “films” of Devaux. For a detailed treatment the reader is referred to excellent reviews that have appeared on spread monolayers of proteins by Rothen (8), Gorter (16), Neurath and Bull (17), Bateman (18), and Bull (19).

THE STRUCTURE OF PROTEIN MOLECULES

Proteins represent a group of rather labile organic macromolecules with a very complicated structure and diversified properties. Despite deficiencies in the understanding of the molecular structure of these substances, many structural aspects have been clearly established. In the natural state, the constituent amino acids have been found to possess a levo-configuration sterically at their $\alpha$-carbon atoms. Moreover, it appears that all proteinaceous materials are constructed from a very small number of these basic units — approximately two dozen have been identified to date. These $\alpha$-amino acids are coupled into complicated polypeptide chains by a repeating peptide linkage between the carboxyl group of one acid and the amino group of another.

In all of the peptide structures determined, the peptide group has been found to be within a few degrees of planarity (20). This is a direct consequence of a partial double-bond character (40%) of the C-N bond (21). In addition, all of the peptide bonds have been found to exhibit a trans configuration for the amide group, that is the $\alpha$-carbon atoms of the polypeptide chain attached to each end of a given planar amide group lie on opposite sides of the C-N bond (20). It is noteworthy that the trans configuration is at least 2 kcal/mole more stable than its cis counterpart (22).
The remaining portions of the amino acids, the amino acid residues, extend as side chains from the α-carbon atoms of this backbone structure. The nature of these residues depends upon the amino acids of which they are a part. In some cases the side chains are uncomplicated aliphatic groups (alanine), for others aromatic or heterocyclic rings (tyrosine, histidine), and still others contain hydroxyl (serine), sulfhydryl (cysteine), or ionizable carboxyl or amino groups (aspartic acid, lysine). The number and arrangement of these groups have an important relationship to the specific properties of a protein.

Another extremely important structural feature of the protein molecule is the three-dimensional arrangement and mode of folding of the peptide chains which determine the nature of the chemical groupings present on its surface. The protein molecule is not composed of an agglomeration of long peptide chains, but follows certain architectural principles by which definite forces operate between adjacent chains to maintain the internal structure of the molecule (23,24).

The form of natural proteins may be fibrous (e.g., silk, myosin), or it may be globular (e.g., albumins, globulins). Astbury and co-workers (25-27) have determined by x-ray technique that in the case of fibrous proteins, the molecules appear to be composed of parallel bundles of polypeptide chains that may assume, reversibly, an α-keratin (relaxed fiber) or β-keratin (stretched fiber) configuration. These parallel polypeptide chains are held together by crosslinking between amino acid residues. These crosslinkages are composed of disulfide bonds, ester linkages, salt linkages, and hydrogen bonds.

The internal structure of globular proteins is considerably more complex and even less clearly understood than that of the fibrous proteins. They are generally spherical or ellipsoidal in shape; their peptide chains are also coiled or folded into well defined patterns which appear to be maintained by the same types of crosslinkage that are present in fibrous molecules. X-ray analysis indicates that denaturation of globular proteins often results in the formation of the β-keratin type of structure (24,28-30). This structure has been confirmed by many investigators.

Pauling and co-workers (31) postulate, from x-ray diffraction data, a structure for proteins that is generally compatible with other data for both the fibrous and the globular type. These authors envision a helical structure in which the peptide units are oriented so that their planes are tangent to an imaginary cylinder around the axis of the helix. Each turn of the helix (360 degrees) contains 3.7 amino acid residues and represents a translation along the axis of 5.4A. The configurational stability of the helix is maintained by hydrogen bonding parallel to the axis, between carbonyl and amino groups that are placed in juxtaposition by the successive turns of the helix. Thus each amide group forms hydrogen bonds with the third amide group from it in either direction along the polypeptide chain (32).

Obviously, the properties of a molecule consisting of closely folded, long peptide chains, such as the one with the helical structure as proposed by Pauling and his associates, would be different than those for a molecule composed of the same constituent peptide chains in the unfolded state such as those existing in protein monolayers.

BEHAVIOR OF PROTEIN MOLECULES AT THE AIR-AQUEOUS INTERFACE

Surface Denaturation

When proteins are spread onto an aqueous surface, or when they diffuse into an interface, partial or total surface denaturation usually occurs as the protein molecules unfold initially into a form which resembles fully extended polypeptide chains of the β-keratin
configuration (28-30). According to Fosbinder and Lessig (33), however, at higher film pressures where the molecules are close packed, the $\alpha$-keratin form may predominate. The spreading process affects the properties of these molecules so that they no longer are those of the native protein. Once the protein has been surface denatured there is slight tendency for it to leave the surface. Surface denaturation occurs very rapidly, and from bulk solution the overall rate of denaturation appears to be a function of the rate of diffusion of the molecules into the interface. This diffusion rate, in turn, is dependent upon the protein concentration and the rate of spreading of the protein on the surface.

Denaturation is brought about by a breakdown of the forces responsible for the integrity of the native protein molecules, which are represented in Pauling's helical protein model by the intramolecular hydrogen bonding along the polypeptide backbone. According to Mirsky and Pauling (34), unfolding of protein molecules is initiated by a loosening of these intramolecular bonds.

With respect to the energy required for this process, the heat of denaturation of a protein has been found to be less than 100 kcal/mole of protein, or a fraction of a kilocalorie per mole of amino acid residues (35). Thus it may be concluded that the structure of the native protein must be such that only a very small energy change is involved in its conversion to the polypeptide configuration at the interface (24). During the process of denaturation hydrogen bonds are both broken and formed, with the final denatured form containing somewhat fewer such bonds than the native molecule (six fewer in trypsin) (24). With 5 kcal/mole as the average bond energy of a protein hydrogen bond, it is improbable that the net energy change during denaturation would ever exceed the 100 kilocalorie maximum observed for the heat of denaturation. Other evidence supporting the mechanism of hydrogen bond cleavage as a prelude to surface denaturation and the interfacial unfolding of a protein is provided by the observation that the unfolding of such molecules may be brought about by such strong hydrogen bonding agents as urea and guanidine hydrochloride (36).

Entropy of Surface Denaturation

The degree of interfacial denaturation may be determined by the entropy changes associated with this process (37). When protein molecules unfold at an air-liquid substrate interface, they are able to assume an extended and variable configuration upon the surface to the extent allowed by various environmental factors, including the type of liquid interface upon which spreading occurs. The entropy of surface denaturation, which is related to the equilibrium between the native and denatured forms of the protein, is a measure of the extent of randomness achieved by the unfolded molecules once the restricting structural bonds have been removed. As might be expected, the entropy of surface denaturation is greater for a given protein spread at an oil-water interface than at an air-water interface. The difference is due to the removal, by the penetrating oil phase, of cohesive van der Waals forces between vertically oriented hydrophobic side chains (38).

If the polypeptide chains are extended at an interface of sufficient area so that they do not interfere with each other, the entropy per peptide chain, which is due to the random motion (twisting and coiling) that takes place as a result of the thermal motion of the substrate, may be expressed as (37)

\[ S_x = (x-2)R \ln(x-1) \]  

or, in terms of each amino acid residue,

\[ s_x = (1 - \frac{2}{x})R \ln(x-1) \]
where $s_x$ is the entropy per each residue, $x$ is the number of such residues in the chain, and $z$ is the coordination number of each residue and will be discussed later in connection with Singer's equation of state for spread protein molecules. For a rigid molecule where $z = 2$ the entropy will be close to zero since, under these conditions of molecular compaction, there is little or no randomness due to bending and coiling of the molecules in the interface. If a protein film is compressed until the polypeptide chains begin to pack together, there will be a concomitant decrease in entropy because of the loss of some of the randomness of motion associated with the expanded state. This decrease in entropy may be obtained from the expression (37)

$$s_x = \int_{A=\infty}^{A} \frac{F dA}{xT} (z = x) - \int_{A=\infty}^{A} \frac{F dA}{xT} (z = 2)$$ (3)

where $F$ is the film pressure, $A$ is the film area, and $T$ is the absolute temperature. The remaining terms are the same as those described in Eq.(2). The second integral represents a correction which must be made to account for the increase in film pressure that would occur due to increased collision frequency of the molecules, even if the molecules were rigid, i.e., $z = 2$.

Protein Solubility and Spreading Tendency

Many proteinaceous materials behave in water like hydrophilic colloids (39). However, as a consequence of surface denaturation and film formation, there occurs a very drastic change in the solubility of these materials, and the films that are formed are insoluble. Some proteins, however, do not spread into monolayers either because of their normally ready solubility or their insolubility in the substrate. Thus it may be concluded that for successful spreading to occur, a protein molecule would have to exhibit solubility characteristics between these two extremes. The prime factor governing the spreading tendency of a protein appears to be the relative number of hydrophilic and hydrophobic groups within the molecule. If the molecule contains a large number of hydrophilic groups, these will render the molecule so soluble that it will be pulled out of the interface into the substrate if it is able to unfold at the interface at all (40). Protamines and gelatin do not form surface films on salt-free water for this reason. In the former case solubility is due to a large number of diamino acids in the structure of the molecule and in the latter, to the large number of glycine residues (41). Conversely, if the protein molecule contains a large number of hydrophobic groups, e.g., leucine and isoleucine residues, spreading will not occur on water because of the complete insolubility of a portion of the molecule. Myosin and fibrinogen are examples of such nonspreading proteins (41).

Thus, it may be said that proteins which can be induced to spread on an aqueous substrate may be characterized by the solubility of some portions of the molecule and the insolubility of others. According to Bull (39) this intramolecular contrast of solubility is a general characteristic of proteins which varies only in degree. The same consideration probably also accounts for the inability of heat-denatured proteins to spread as readily as native ones, since it appears that the hydrophobicity of a denatured protein molecule is increased to a considerable extent over that of its native counterpart (40). The increase is thought to occur through: (a) combination, within the molecule, of the carboxyl and amino groups, which were previously free to bind with the substrate, into an amide-like linkage which would have less attraction for water, and (b) by a strengthening of the intramolecular cohesive forces which make the protein molecules more difficult to unfold at the surface (42).
Lack of spreading of a protein may be due, however, to causes other than that of insolubility of a large portion of the molecule. Scleroproteins, for example, fail to form monolayers because of the presence of intramolecular forces of cohesion, similar to those occurring as the result of heat denaturation. These forces are too strong to be overcome by the forces which bring about the spreading of other proteins into monomolecular films (34). In other words the work of cohesion in scleroproteins is greater than the work of adhesion.

In the examples of nonspreading proteins given above, pure water was used as the substrate. In many instances proteins that spread poorly or not at all on water may be made to spread completely by the addition of a suitable electrolyte to the substrate. Thus the highly soluble gelatin molecule can be induced to spread on ammonium sulfate solutions. In the case of fibrinogen and myosin and other insoluble protein molecules, spreading may be achieved by subjecting them first to limited tryptic or peptic digestion (41,43). Continued proteolytic digestion will reduce the protein molecule to soluble fragments.

Hydration of hydrophilic groups of protein molecules by an aqueous substrate plays an important role in the mechanism of film formation. In this way the protein film becomes anchored to the substrate, via hydrogen bonding between the hydrophilic groups and the water, with the molecules assuming a definite orientation with respect to the interface (42). These hydrophilic groups are divided into two general classes (44). They are the polar side chains (amino acid residues) such as those in aspartic acid and lysine, and the oxygen and nitrogen of the peptide bonds. One would expect each of the hydration centers (=O and >N-H) of the polypeptide backbone to be capable of binding two molecules of water; however, because of steric considerations and the loss of some of the hydration centers to intramolecular bonding, one water molecule per hydration center is probably too high an estimate. During the course of monolayer formation protein molecules unfold so that hydrophilic ionic groups are oriented toward the aqueous substrate, while nonpolar hydrophobic groups are oriented upward from the peptide backbone toward the air phase of the interface (17,23). As will be discussed later, it may be that the initial position of the nonpolar side chains is such that they lay in the plane of the interface, as far as it is sterically possible, when the protein is present on the substrate at low concentration.

Insolubility of Protein Films

To reiterate, the unfolding of a protein molecule is accompanied by an enormous diminution in its solubility, a fact which may be readily shown by application of the Gibbs adsorption equation (45),

\[ dF = \sigma \ kT \ d\ln C \]  

where \( C \) is the concentration of the protein in the substrate, \( \sigma \) is the number of molecules in the adsorbed film per cm\(^2\), \( k \) is the Boltzman constant (1.37 × 10\(^{-16}\) erg/deg), and \( F \) is the surface pressure in dynes/cm. The solubility of the molecules making up an adsorbed film, such as a protein monolayer, should increase rapidly if the film is subjected to an increasing surface pressure. According to calculations presented by Langmuir, if a film of a protein with a molecular weight of 35,000 and a specific area of 1 m\(^2\)/mg is compressed so that the surface pressure is increased by 1 dyne/cm, the solubility of the film molecules should increase by a factor of 10\(^6\). The application of the Gibbs equation is independent of the molecular weight of the protein since the units used in measuring the surface concentration and bulk concentration of the solute must be the same. Since no increase in film solubility has even been observed during the course of film compression, even at high film pressures or during actual film collapse, it may be concluded that protein.
molecules form an insoluble layer at the air-substrate interface. This extreme insolubility of protein films even at high film pressure seems to indicate that compression does not refold the molecules to their original nondenatured state - if such were the case the molecules would regain at least some of their original solubility characteristics. The decrease in solubility as a result of the unfolding of the molecules on the surface accounts for the ability of a protein to form insoluble monomolecular films while being soluble in the globular state.

The high solubility of many proteins in water indicates that nearly all of the surface of the molecules is hydrophilic in nature. Hydrophobic groups will tend, as far as it is possible sterically, to escape from the water into the interior of the molecule, where through the action of strong cohesive forces, they will bind the molecule tightly together (38). Unfolding of the molecules uncovers these hydrophobic groups (42); for unfolding to occur the spreading forces must overcome this internal cohesion.

Orientation of Film Molecules and Film Compression

Orientation of protein molecules on the surface is governed by the number of hydration centers exposed to the aqueous substrate, distribution of the various kinds of amino acids in the chain, and surface pressure. The fairly rigid planar amide groups of the polypeptide backbone may or may not be able to rotate relative to each other depending upon the nature of the side chains attached to the \( \alpha \) -carbon atoms (20). Assuming no steric hindrance of this nature, the minimum potential energy of a protein film will occur when the whole structure is lying flat on the surface of the substrate; the main anchorage between film and substrate is provided by the strongly polar free carboxyl and amino groups of the various amino acid residues and the peptide bonds of the backbone structure (29,46,47).

The arrangement of the polypeptide chains at the interface will also vary according to the degree of lateral compression to which they may be subjected. According to Hughes and Rideal (29) and Ellis and Pankhurst (48), the following sequence of events probably occurs during the compression of a protein monolayer. In the expanded state, the amino acid residues, including the relatively nonpolar ones, lie flat on the surface if given the opportunity, and no film pressure will be obtained until contact is made between residues of neighboring chains. The precise nature of the expanded state, however, does not seem to be clearly understood. According to Davies (37) the protein molecule at the air-water interface is folded back on itself in two dimensions due to the cohesive forces acting between the nonpolar side chains. Such binding within the molecule acts to severely restrict the extension of the molecule on the substrate surface. At an oil-water interface most of this cohesive binding is lost and the molecules can assume a more nearly random configuration and thus become more extended.

Compression of the expanded film causes the amino acid residues of adjacent peptide chains to interlock to give a close-packed film with the side chains still lying flat. This is called the "condensed phase" of the film. If the film is compressed beyond this point, an alteration in the film structure occurs. The hydrophobic side chains begin to assume a position in the air phase more nearly normal to the interface to the extent that steric considerations permit, while the chains with polar heads tilt downward. Thus a gelation of the film occurs. In this state the film is still a monolayer with respect to the polypeptide backbone, but the mean thickness of the monolayer has increased. Rigidity of the film in this state is enhanced by mutual attraction of close-packed polypeptide chains and by cohesive forces between close-packed hydrocarbon side chains. X-ray data indicate (29) that the polypeptide chains approach each other to approximately 4.5 Å at this stage of compression. It is postulated (29) that chemical linkage between adjacent peptide groups may take place due to "secondary valencies," since under prolonged compression, the film becomes irreversible in expansion with respect to both surface potential and film pressure.
Intrafilm Forces

According to Davies and Llopis (49), there exist several different types of interacting forces in a spread film. While much of their work was carried out on simpler peptide systems composed of polyamino acids and in the low pressure region, the results obtained for these simpler systems seem to be generally applicable to proteins.

In the case of a synthetic polypeptide such as polyalanine or polyleucine, or a protein containing a high percentage of nonpolar amino acid residues, the polypeptide chain as spread on the surface will remain bunched up because of cohesive forces between the hydrophobic side chains (37). The stabilizing effect of the coalescence of such hydrophobic groups has been estimated to be about 2 kcal/mole per CH₂ group (24). If such a molecule is unfolded at an oil-water interface, these intramolecular cohesive forces will be lost because of the penetration of oil between the alkyl side chains. This will cause the molecule to unfold into the most nearly random configuration consistent with steric limitations (39).

The presence of a net electric charge, involving some or all of the residues, will lead to a partial or complete unfolding of the protein molecule as a result of intramolecular repulsion. This force lowers the energy barrier set up by the van der Waals cohesive forces and allows the molecules to spread out by the action of the thermal motion of the substrate water molecules. This may be expressed as:

\[ z - 2 = \omega = \omega_0 \exp \left( \frac{E}{kT} \right) \]  

where \( \omega \) is a measure of the flexibility of the molecules in the interface and \( \omega_0 \) is the value of \( \omega \) when the van der Waals energy barrier \( E \) is zero (no internal cohesion). The coordination number \( (z) \) of each amine acid residue will be discussed below in connection with Singer's equation. Both of these internal interactions are directly responsible for altering the flexibility of the peptide chains in the surface.

A third type of interaction is that of cohesion and/or repulsion between different molecules in the surface. Intermolecular cohesive forces have been found to be unimportant under certain conditions for monolayers of proteins where molecular separation is large (49). Thus at or near the isoelectric point or on strong salt solutions, Bull (19) has found that FA-vs-F plots are practically linear over a wide range of pressures in the low pressure region (50). If a rearranged form of the equation of Volmer and Mahnert (51),

\[ FA = kT + Fb - a \left( \frac{1}{A} - \frac{b}{A^2} \right) \]  

is considered, it can be seen why intermolecular cohesion is low. In this equation \( a \) represents the intermolecular interaction and \( b \) is a constant proportional to the area occupied by the close-packed molecules on the surface. For linear FA-vs-F plots \( a \) must be practically zero. There is, however, a slight intermolecular cohesion above a film pressure of the order of 0.1 dyne/cm for many proteins. Bull (44) has found that at this low pressure there exists a small plateau in the FA-vs-F plot. The slope of the curve, which is indicative of the freedom of the molecules in the monolayer, shows that the film molecules are less restricted at this pressure and below. In films where the protein molecules have been compacted sufficiently, intermolecular cohesion may be determined by measuring surface viscosity and elasticity. Some protein films have been found to exhibit no viscoelastic behavior until the molecules have been compressed to such an extent that they are in physical contact. According to Hughes and Rideal (29), this is the stage of compression at which the polar side chains are becoming immersed in the substrate and rigidity of the film occurs as a result of the cohesive forces between the close-packed nonpolar side chains.
Where molecular separation is great, intermolecular repulsion has little effect. Even at slight compression the effect is rather small, at least in the cases of hemoglobin, or albumin and related proteins on an acid substrate (38). Cheesman and Davies conclude that the ionized groups and their neighbors must be pulled into the bulk of the substrate, thereby reducing any lateral electrical effect.

By applying the results of the work on high polymers by Flory (52) and Huggins (53) to the study of protein monolayers, Singer (54) has been able to show a mathematical relationship between the pressure of a film (in the low pressure region) at a given area as a function of several film parameters. This treatment, which is valid for uncharged films at both the air-water and oil-water interface, may be expressed as

\[ F = -\frac{kT}{A_0} \left[ \ln \left(1 - \frac{A_0}{A}\right) - \frac{(x-1)z}{2} \ln \left(1 - \frac{2}{z} \frac{A_0}{A}\right) \right] \]  (7)

where \( x \) is the degree of polymerization, i.e., the number of amino acid residues, \( A \) is the available substrate area per amino acid residue, \( A_0 \) is the area of each residue in the close-packed state, and \( z \) is a coordination number relating to the quasi-lattice of the amino acid residues in the interface. The coordination number is a quantitative representation of the steric configuration of neighboring amino acid units about any other given amino acid unit in the polypeptide chain. If the film molecules are completely rigid, each amino acid unit will have only two positions, one on each side, to which other amino acid units can be fitted. For the rigid molecule there is no flexibility in the polypeptide chains and \( z \) is, accordingly, assigned the value of 2. If the chain is completely flexible so that each unit in the chain could bend at right angles to its neighbor, the value of \( z \) becomes 4. The amount by which \( z \) exceeds 2 is a measure of the flexibility of the polypeptide chain and is designated as \( \omega \) (Eq. (5)) so that \( \omega = z - 2 \) (37). In the case of the rigid molecule, \( \omega = 0 \), i.e., no flexibility. Also under this condition Singer's equation will reduce to

\[ F = \frac{-kT}{xA_0} \ln \left(1 - \frac{A_0}{A}\right). \]  (8)

At very high film areas in which the ratio \( A/A_0 \) becomes large (\( F \rightarrow 0 \)), the Singer equation reduces to \( F = kT/xA \), irrespective of the value of the coordination number.

The foregoing discussion does not present an all inclusive picture of the behavior of proteinaceous materials as they are extended at a suitable interface. In addition, how these surface properties, observed from a study of films formed in an artificial environment, compare to those possessed by films of proteins native to a cell is indeterminable. However, it is not unreasonable to assume that the more basic mechanisms of film formation are valid in both instances. Certainly a prime target for investigation would be the mechanism by which proteins are apparently able to undergo reversible film formation within the cell. In this instance native and artificial film properties appear to be at variance. Work with enzymes has indicated, by loss of enzymic activity, that in the formation of artificial films the protein undergoes an irreversible surface denaturation. Also, as stated earlier, films formed under laboratory conditions are extremely insoluble.

It is probable that film formation at intracellular interfaces is not complete in the sense that the proteinaceous material does not unfold to the extent that it does under laboratory conditions. For a partial unfolding of the protein to occur the surface forces responsible for normal spreading would have to be regulated. The energy for such a mechanism could be provided by the metabolic energy pool present in living tissue which maintains cellular life processes in a state remote from equilibrium.
SUMMARY

The importance of the role assumed by monomolecular films of proteins and other biological materials in supporting the metabolic functions of a living cell has been more fully recognized in recent years. It is, therefore, pertinent that a study be made of these materials as they exist in this physical state, so that a better understanding may be had as to how these films are formed and maintained, and how they function within the cell.
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The role played by monomolecular films of proteins and other biological materials in supporting the metabolic functions of a living cell has become more fully recognized in recent years. Thus the relevance of a study of films of these materials in the laboratory in an effort to determine their method of formation, nature, and mode of action within the cell becomes obvious. As a brief introduction to this area of research, a general review relating to the surface properties of proteins is presented. This includes discussion of the structure of protein molecules, surface properties, and the role of these films in biological systems.
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