Biochem. J. (1987) 244, 295–301 (Printed in Great Britain)

Interactions of ovalbumin and of its putative signal sequence with phospholipid monolayers

Possible importance of differing lateral stabilities in protein translocation

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Surface properties of ovalbumin and of its putative signal sequence, and their interactions with phospholipids at an air–water interface, have been studied. The mature protein can form an interfacial film spontaneously from its bulk solution, whereas the signal sequence cannot. Mature ovalbumin also penetrates phospholipid monolayers from the subphase (independently of the type of phospholipid present), whereas its signal sequence does not. The surface stability of a spread film of the signal sequence is, however, higher than that of a film of mature ovalbumin. Above specific threshold concentrations of signal peptide and of mature ovalbumin in mixed films with phospholipids, two separate phases are formed. In such immiscible films, the signal sequence peptide is also able to support a higher lateral surface pressure than mature ovalbumin, at corresponding areas of peptide and mature protein in the mixed monolayers. It is suggested that the differing lateral stabilities of ovalbumin and of its putative signal sequence may be relevant to the translocation of ovalbumin across the membrane of the endoplasmic reticulum, and a scheme for its translocation is proposed that is based on these properties.

INTRODUCTION

Ovalbumin is the major secretory protein from hen oviduct, and it represents an exception to the general observation that exported proteins are initially biosynthesized with a transient *N*-terminal pre-piece (Palmiter *et al.* 1978). According to the signal hypothesis, this additional peptide sequence is necessary for the cotranslational, vectorial transport of proteins through rough endoplasmic reticulum membranes, which are thought to contain complex structures such as hydrophilic tunnels through which nascent chains translocate (Blobel & Dobberstein, 1975), the signal peptide being targeted to the membrane by a ribonucleoprotein particle (signal recognition particle) (Walter & Blobel, 1980; Meyer *et al.*, 1982). To explain the absence of a cleaved *N*-terminal signal sequence from ovalbumin, it has been proposed that the mature protein contains a functionally equivalent signal sequence that is not proteolytically removed in the lumen of the endoplasmic reticulum. It has also been suggested that a sequence of about 20 uncharged residues, which commences at the 27th amino acid and has a high index of hydrophobicity, might be involved (McReynolds *et al.*, 1978; Austen, 1979). This proposal was supported by studies which showed that a functionally equivalent signal sequence of ovalbumin is apparently located within the first 60 residues of the *N*-terminus (Meek *et al.*, 1982). Using mutant ovalbumin proteins, Tabe *et al.* (1984) then concluded that an internal signal sequence is located within amino acids 22–41 rather than between residues 234 and 253 as proposed by Lingappa *et al.* (1979). The spontaneous insertion models of protein translocation (von Heijne & Blomberg, 1979; Wickner, 1980; Engelman & Steitz, 1981) may also account for the secretion of ovalbumin.

The several models that have been proposed for protein translocation (including the signal hypothesis) take no account of the dynamic structure of membranes (cf. Chapman & Benga, 1984), and roles that have been suggested for specific membrane phospholipids, e.g. acidic phospholipids in the bacterial cytoplasmic membrane (Nesmeyanova, 1982), are not generally applicable to the translocation process in eukaryotes. The extent to which nascent protein chains are exposed to a lipid environment during their passage through membranes is not clear. However, as the signal pre-piece or its internal equivalent crosses an anisotropic hydrophobic–hydrophilic barrier of phospholipids during translocation, the interfacial properties of signal sequence peptides (as well as their conformational properties; Austen & Ridd, 1981; Emr & Silhavy, 1983) could well be important. In this connection, we recently reported that the surface stabilities of three signal sequence peptides (pretrypsinogen 2, a 'consensus' signal sequence and the putative signal sequence of ovalbumin) are higher than those of proteins and polypeptides which were studied previously (Fidelio *et al.*, 1986*a*). In the present paper, we extend these observations by describing the interactions of ovalbumin and of its putative signal sequence with phospholipids at an air–water interface. In the light of this work, a dynamic model for protein translocation is proposed in which the properties of lipid/protein

Abbreviations used: DIOLPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; DIOLPG, dioleoylphosphatidylglycerol; G_{M1}, Gal(β1→3)GalNAc(β1-4)Gal[(3←2α)NeuAc]-(β1→4)Glc(β1→1)Cer (*N*-acylsphingoid).

mixtures, and the lateral stabilities of the signal sequence and of the mature protein at the interface, play important roles in the translocation process.

MATERIALS AND METHODS

Details of the monolayer equipment used, procedures for penetration studies, and the preparation of single and mixed lipid/protein films, have been given previously (Maggio & Lucy, 1975; Fidelio *et al.*, 1982, 1984, 1986*a*). The lipids and ovalbumin were from Sigma, and the purity of the phospholipids was checked by h.p.t.l.c. G_{M1} ganglioside was treated as described previously (Fidelio *et al.*, 1982). The phospholipids were dissolved in chloroform/methanol (2:1, v/v) and G_{M1} was stored in chloroform/methanol/0.1 M-NaOH (40:20:1, by vol.). For the preparation of the mixed films, ovalbumin and the signal sequences were stored in chloroform/methanol/water (8:8:1, by vol.) and chloroform/methanol/dimethyl sulphoxide (3:3:2, by vol.) respectively. For the penetration experiments, the proteins were stored in water. All the solvents used were AristaR grade from BDH.

The putative signal sequence in a peptide comprising residues 21–47 of ovalbumin was isolated from a tryptic digest of the protein, and it was used in the *S*-sulphonylated ($S-SO_3^-$) form, the reduced form (–SH), or the *S*-carboxyamidomethylated ($-CH_2CONH_2$) form (Robinson *et al.*, 1986). The sulphonylated form was used in the experiments with mixed lipid/signal peptide films, and its concentration was checked by amino acid analyses after hydrolysis. All experiments were carried out at 22±1 °C on a subphase of unbuffered 145 mM-NaCl. The pH was adjusted to 6.5–7.0 with HCl (2 M) or NaOH (2 M); there were no differences in the results obtained between pH 5 and pH 7. To investigate mixed lipid/protein films, solutions of the individual components were pre-mixed before films were spread. The behaviour of mixed monolayers was analysed by comparing the force–area curves and surface potential–area curves obtained experimentally with ideal, theoretical isotherms for the films (Gaines, 1966; Maggio *et al.*, 1978*b*). Immiscible behaviour between film-forming molecules was evaluated according to the surface phase rule (cf. Gaines, 1966). All experiments were done at least in duplicate: they were reproducible with ±1 $mN \cdot m^{-1}$ for surface pressure and within ±5% for surface potential and molecular areas.

RESULTS

Interfacial behaviour of single components

The surface pressure–area and surface potential–area isotherms for the reduced, sulphonylated and carboxyamidomethylated forms of the putative signal sequence of ovalbumin are very similar (Fig. 1), indicating that the chemical differences between these derivatives do not markedly affect their surface behaviour. The minimum molecular areas (limiting areas) found were between 2.0 and 2.2 nm^2 (Fig. 1). Previous studies indicated values of 1.4 and 1.6 nm^2 for these parameters (Fidelio *et al.*, 1986*a*). Such values are consistent with a considerable degree of secondary structure at the surface, such as an α-helix perpendicular to the interface or a 'loop' structure in which two antiparallel β-strands are linked by a β-turn. The observed collapse pressures for the signal sequence (the maximum lateral pressure that films can sustain before disruption of the monomolecular arrangement) were between 26 and 31 $mN \cdot m^{-1}$ (Fig. 1). These pressures are, on average, 7–8 $mN \cdot m^{-1}$ higher than the collapse pressures of other proteins and even of the highly amphipathic basic polypeptide, melittin (Fidelio *et al.*, 1984).

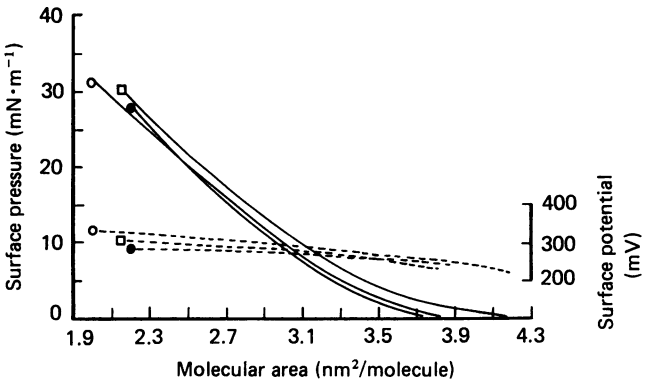

Fig. 1. Surface isotherms of the ovalbumin signal sequence peptide

Surface pressure (——) and surface potential (----) isotherms of *S*-sulphonylated (●), reduced (□), and *S*-carboxymethylated (○) forms of the ovalbumin signal sequence. Subphase 145 mM-NaCl at pH 7.

By contrast, the collapse pressure achieved by mature ovalbumin is only 20 $mN \cdot m^{-1}$ (Fig. 2*a*), and this value is similar to that obtained for other proteins (cf. Fidelio

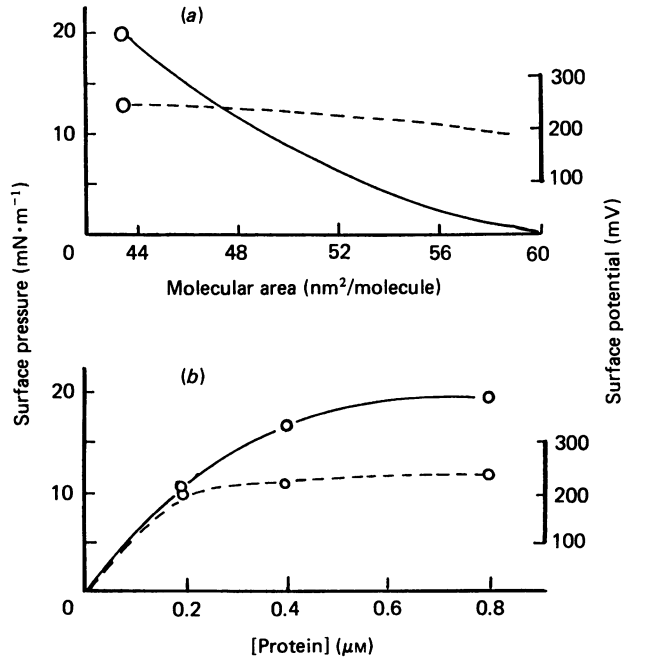

Fig. 2. Surface behaviour of ovalbumin at lipid-free interfaces

(*a*) Surface pressure (——) and surface potential (---) isotherms of ovalbumin spread at the interface. (*b*) Surface pressures (——) and surface potentials (---) observed after the injection of ovalbumin into the subphase at the concentrations indicated. Subphase as in Fig. 1.

et al., 1986*a*). Mature ovalbumin thus has a lower surface stability than its signal sequence. The limiting molecular area found for mature ovalbumin, about 43 nm^2 (Fig. 2*a*), is approximately twice the value that would be expected for a compact spherical shape of the same mass in solution. It should be emphasized, however, that data on the cross-section of a protein at the interface need not necessarily coincide with that obtained from bulk solutions, since thermodynamic restrictions on the conformation of a protein in the surface may be different from those prevailing in solution. In solution, variations in hydrophobicity in the primary structure of a soluble protein result in a partitioning of the molecule into structural segments, which interact to form a hydrophobic core (Rose & Siddhartha, 1980). At the surface, a soluble protein has to accommodate its complex hydrophobic profile (Rose & Siddharta, 1980) in a more expanded conformation, as discussed previously for bovine serum albumin by Fidelio *et al.* (1984).

The sulphonylated form of the ovalbumin signal peptide is unable to partition to a clean interface from the most concentrated solution (1 μM) of the peptide that can be obtained in the subphase (Fidelio *et al.*, 1986*a*). When mature ovalbumin is injected into the subphase beneath a clean interface, however, the surface pressure and surface potential increase with the concentration of the protein up to a limiting concentration of 0.8 μM (Fig. 2*b*). The maximum values of surface pressure (termed the adsorption equilibrium surface pressure) and of surface potential obtained in this way are approximately equal to those seen at the limiting molecular area of spread films of the protein (Fig. 2*a*). This indicates that the surface properties of ovalbumin films are independent of whether they are prepared by adsorption or by spreading (cf. MacRitchie, 1978; Fidelio *et al.*, 1982).

To summarize the interfacial properties of the putative signal sequence and of the mature protein, our experiments show that spread films of the signal sequence can support higher collapse pressures than films of mature ovalbumin. Conversely, unlikely the mature protein, the signal sequence cannot form a surface film spontaneously from its bulk solution.

Penetration into lipid monolayers

The putative signal sequence is also unable to penetrate from the bulk phase into monolayers of lipid, and it does not increase their surface pressure. By contrast, increases in surface pressure are induced by the injection of mature ovalbumin (at a concentration of 0.8 μM) into the subphase beneath seven different lipids at an initial surface pressure of 10 $mN \cdot m^{-1}$ (Figs. 3 and 4). These increases occur rapidly and they reach 60–80% of the final values in 5–10 min (results not shown). The final values of surface pressure for the lipid/protein films (17–21 $mN \cdot m^{-1}$) are close to those which have been found in similar experiments on the interactions of films of bovine serum albumin, myelin basic protein, glycophorin and melittin with neutral and acidic phospholipids (Fidelio *et al.*, 1981, 1982). It is apparent from our data that ovalbumin does not interact preferentially with any particular lipid. Furthermore, the final values of surface pressure are not dependent on a particular physical state of the interface, since ovalbumin interacts similarly with liquid-condensed DPPC, with liquid-expanded DMPC and DIOLPC (cf. Phillips, 1972), and

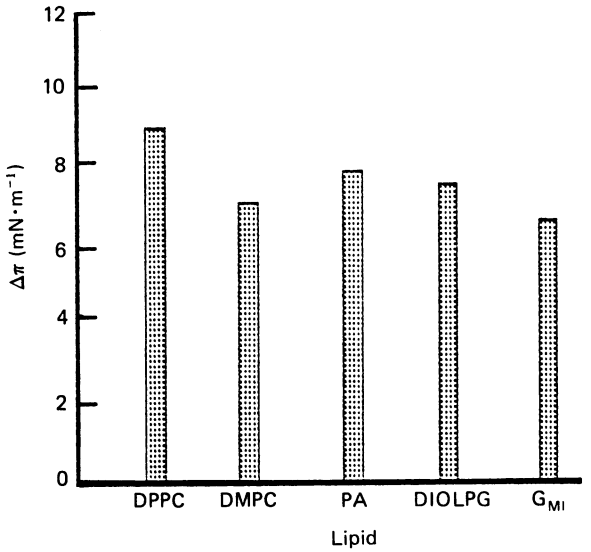

Fig. 3. Effects of lipid–ovalbumin interactions on surface pressure

Increments in surface pressure ($\Delta\pi$) induced by the injection of ovalbumin (0.8 μM) into the subphase beneath monolayers of lipids at an initial surface pressure of 10 $mN \cdot m^{-1}$. Subphase as in Fig. 1.

with the complex liquid-expanded glycosphingolipid G_{M1} (cf. Maggio *et al.*, 1978*a*).

The penetration of ovalbumin into lipid films is surface-pressure-dependent, and the well-known decrease in penetration with increasing surface pressure of the initial lipid film (Quinn & Dawson, 1969) is observed for the penetration of ovalbumin into DIOLPC and PE (Fig. 4). It is noteworthy that, as observed for other lipid/protein systems (Fidelio *et al.*, 1982, 1986*b*), ovalbumin is able to interact with lipids to produce an increment of about 4–5 $mN \cdot m^{-1}$ in surface pressure even when the initial surface pressure is equal to its own collapse pressure. This indicates that the surface properties of ovalbumin are modified by its interaction

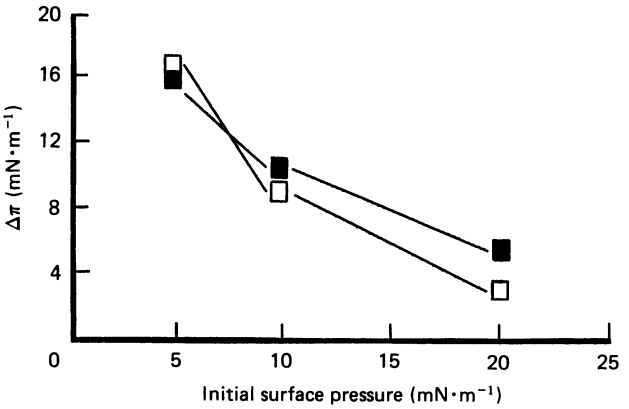

Fig. 4. Effects of the initial surface pressure of phospholipids on ovalbumin–phospholipid interactions

Increments of surface pressure ($\Delta\pi$) induced by the injection of ovalbumin (0.8 μM) beneath monolayers of DIOLPC (□) and of PE (■) at the initial surface pressures indicated. Subphase as in Fig. 1.

with lipids in such a way as to enable it to support a higher lateral pressure.

Changes in surface potential after injection of ovalbumin into the subphase beneath the seven different lipids studied were found to follow the same time course as the surface pressure changes, and the final increments were between 5 and 40 mV (results not shown).

Interfacial behaviour of spread mixed lipid/protein films

The sulphonylated form of the ovalbumin signal peptide is apparently miscible with both DIOLPC and PE at molar fractions of peptide that are less than 0.2, and the mixed films that are formed have a single collapse pressure of 43–46 $mN \cdot m^{-1}$ (which is similar to that of the phospholipid components alone). Similar, single collapse pressures are exhibited by homogenous mixed films of mature ovalbumin with DIOLPC and PE at molar fractions of protein that are less than 0.0025 and 0.005 respectively.

For both one-component and mixed monolayers, the collapse pressure represents the maximum surface pressure that can be sustained before the film is transformed into a bulk phase at the interface or some of the film is squeezed out into the subphase. While the collapse pressures of mixed homogenous films vary with their composition, the surface phase rule requires that immiscible components in a mixed film collapse independently (cf. Gaines, 1966; Maggio *et al.*, 1978*b*) and, in some cases, the formation of a collapsed phase at the interface has been directly demonstrated by electron microscopy for lipid/protein (Gabrielli & Baglioni, 1981) and lipid/lipid (Ries & Swift, 1982) mixtures. Lateral immiscibility can occur abruptly in mixed lipid/protein films when the protein concentration at the surface exceeds a critical value (Fidelio *et al.*, 1984). For convenience, the behaviour of mature ovalbumin will be considered first in this connection. We have observed that mixed films of ovalbumin/DIOLPC and ovalbumin/PE exhibit lateral immiscibility (cf. the development of inflexions in the pressure–area curves of Fig. 5) when the molar fractions of protein reach 0.0025 and 0.005 respectively (Fig. 6). Two collapse points are then observed and, for concentrations of protein near to the critical value, the surface pressure at the lower of these points (the collapse pressure of the protein-rich component) is 27–30 $mN \cdot m^{-1}$. Since this is 7–10 $mN \cdot m^{-1}$ greater than the collapse pressure of an ovalbumin film (20 $mN \cdot m^{-1}$) (compare Figs. 6*a* and 6*c* with Fig. 2*a*), it appears that ovalbumin has a greater lateral stability in the presence of the lipid, or that a lipid–protein complex is formed which is more stable than the pure protein (cf. Fidelio *et al.*, 1984, 1986*b*).

With DIOLPC up to about 14%, and with PE up to about 27%, of the area of a mixed film can be occupied by ovalbumin before two collapse points are observed as the two components become immiscible (Figs. 6*a* and 6*c*). At higher concentrations of protein, the collapse pressure of the immiscible ovalbumin component decreases to values that are less than 30 $mN \cdot m^{-1}$ (see Fig. 5 and the shaded regions of Fig. 6*a* and 6*c*).

In mixed films of the signal sequence with DIOLPC, the peptide can occupy up to about 31% of the monolayer area (and up to about 46% with PE) before lateral immiscibility develops (Fig. 7*a* and 7*c*). For concentrations of peptide that are near to the critical value, the collapse pressure of the peptide component in the immiscible films is 38–41 $mN \cdot m^{-1}$, and a comparison of Figs. 7(*a*) and 7(*c*) with Figs. 6(*a*) and 6(*c*) shows that (for corresponding areas of signal peptide and mature ovalbumin in the mixed monolayers) the signal sequence peptide can support a higher lateral surface pressure (by at least 10 $mN \cdot m^{-1}$) than mature ovalbumin. The collapse pessure of the signal peptide is thus higher than the collapse pressure of mature ovalbumin in mixed films with phospholipids as well as in single component monolayers.

In addition, at their (lower) collapse pressures, mixed films of the signal sequence with phospholipids exhibit

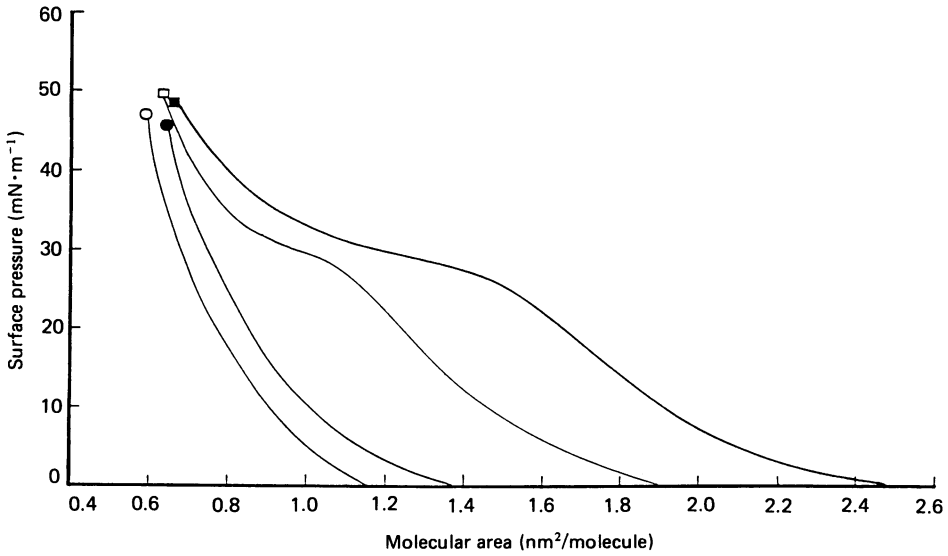

Fig. 5. Surface behaviour of mixed monolayers of ovalbumin and PE

Surface pressure–area isotherms of mixed monolayers of ovalbumin and PE for protein:lipid molar fractions of 0:1 (○), 0.0027:0.9973 (●), 0.01:0.99 (□) and 0.02:0.98 (■). The two isotherms on the right (biphasic systems) show two collapse points (the lower of which is termed the first discontinuity of the isotherm). Subphase as in Fig. 1.

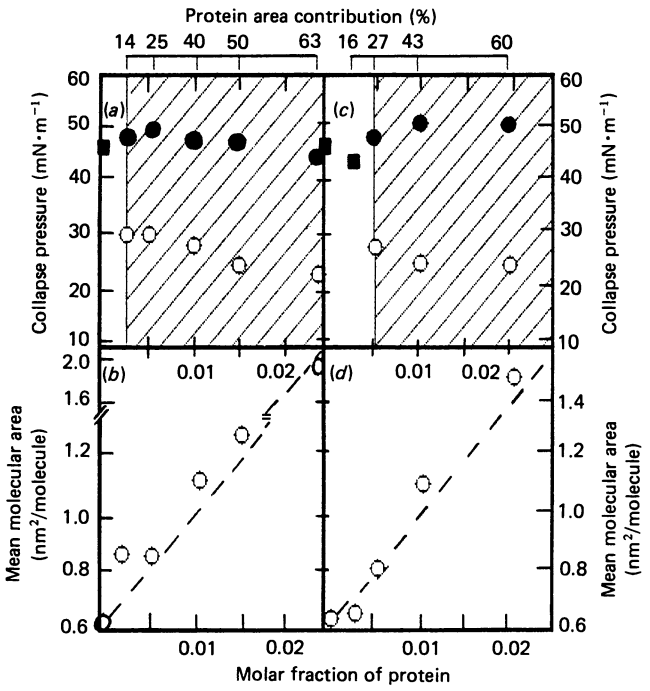

Fig. 6. Surface behaviour of ovalbumin–phospholipid interfaces

(*a*) Collapse pressures for monolayers of ovalbumin mixed with DIOLPC, and (*c*) with PE. The single collapse pressure (■) for the monophasic systems, and the lower (○) and higher (●) collapse points for the biphasic systems (shaded regions) are shown. (*b*) Molecular areas for monolayers of ovalbumin mixed with DIOLPC, and (*d*) with PE. Dashed lines indicate the molecular areas expected for ideal mixing, and (○) represents the experimental values found for the mean molecular areas at the (lower) collapse points. The area (%) of the mixed film that is occupied by protein is calculated from the molecular area occupied by the protein alone, and the molar fraction of protein present in the mixed film. Subphase as in Fig. 1.

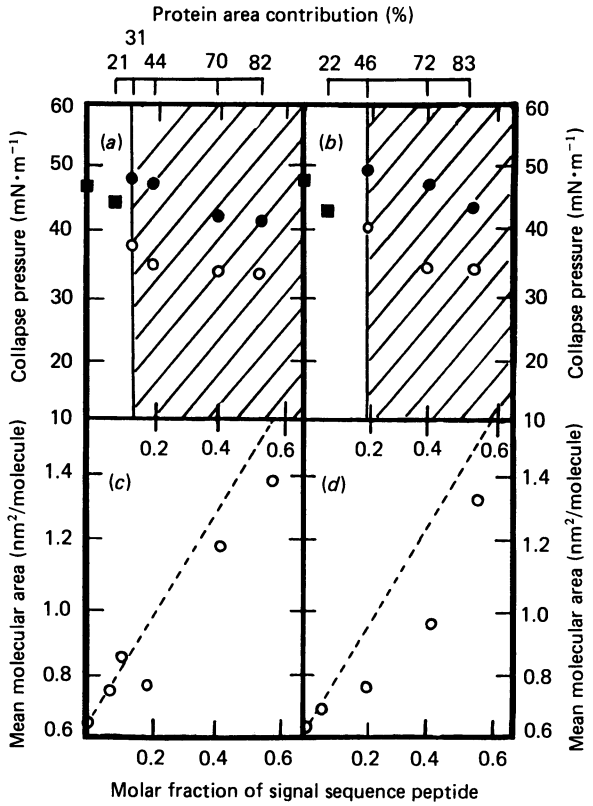

Fig. 7. Surface behaviour of *S*-sulphonylated ovalbumin signal sequence peptide–phospholipid interfaces

(*a*) Collapse pressures for monolayers of the *S*-sulphonylated ovalbumin signal sequence mixed with DIOLPC, and (*c*) with PE. The single collapse pressure (■) for the monophasic systems, and the lower (○) and higher (●) collapse points for the biphasic systems (shaded regions) are shown. (*b*) Molecular areas for monolayers of the signal sequence mixed with DIOLPC, and (*d*) with PE. Broken lines indicate the molecular area expected for ideal mixing, and (○) represents the experimental values found for the mean molecular areas at the (lower) collapse points. The area (%) of the mixed film that is occupied by the signal peptide is calculated from the molecular area occupied by the peptide alone, and the molar fraction of peptide present in the mixed film. Subphase as in Fig. 1.

decreases in mean molecular area, by comparison with ideal mixing behaviour, which do not occur in mixed films of mature ovalbumin with phospholipids (compare Figs. 7*b* and 7*d* with Figs. 6*b* and 6*d*). One possible explanation of this phenomenon is that the signal sequence peptide changes its conformation, in the presence of phospholipid molecules at this pressure, from an α-helix or a looped β-structure to an extended β-structure which is perpendicular to the interface and has a molecular area of about 0.7 nm^2 (assuming an average length of amino acid side chain of 0.5 nm).

Inspection of the collapse pressures of the lipid components of immiscible ovalbumin/phospholipid and signal peptide/phospholipid mixtures reveals that, at the point of immiscibility, the lipid-rich phases exhibit rather higher collapse pressures (48–50 $mN \cdot m^{-1}$) than the corresponding pure phospholipids (46 $mN \cdot m^{-1}$ for both DIOLPC and for PE). Increases in the concentration of mature ovalbumin then have little effect on the collapse pressures of the lipid components in the immiscible mixed films (Figs. 6*a* and 6*c*), but increases in the concentration of signal peptide cause the collapse pressures of the lipid-rich phases to fall (Figs. 7*a* and 7*c*). These findings indicate that the properties and lateral stabilities of the lipids are modified by the protein–lipid interactions that occur in these systems as well as the behaviour of ovalbumin and of its signal peptide being modified by the presence of phospholipids (cf. Fidelio *et al.*, 1982, 1984).

DISCUSSION

It is of interest to consider the possible implications of our findings for the translocation of proteins across endoplasmic reticulum membranes. There is indirect evidence that the surface pressures of natural membranes are between 20 and 35 $mN \cdot m^{-1}$ (Bangham, 1968; van Deenen *et al.*, 1976; Israelachvili *et al.*, 1980). When, in preliminary work, we initially observed that the collapse pressure of mature ovalbumin is only 20 $mN \cdot m^{-1}$ (Fidelio *et al.*, 1986*c*), whereas that of its putative signal sequence is 26.5 $mN \cdot m^{-1}$ (Fidelio *et al.*, 1986*a*), two

suggestions were therefore put forward (Fidelio *et al.*, 1986*c*).

Firstly, it was proposed that the ability of signal peptides to support a high lateral surface pressure may facilitate the binding of the nascent chain–polysome complex to the endoplasmic reticulum at the beginning of the translocation process. The unexpected inability of the putative signal peptide of ovalbumin to penetrate from the bulk phase into monolayers of phospholipid, which we have now observed, may have resulted from aggregation of the peptide in the bulk phase. It is, however, also consistent with the hypothesis that the initial entry of a signal peptide into endoplasmic reticulum membranes involves its interaction with a specific membrane-bound system, such as the signal-recognition machinery that has recently been postulated (Rothman & Kornberg, 1986) to provide specificity for the initial entry of proteins into membranes and catalyse the unfolding of proteins which may be necessary for their translocation (Eilers & Schatz, 1986). By contrast, no signal recognition machinery has been identified or isolated from bacteria (Silhavy *et al.*, 1983). Translocation in bacteria appears also to be less tightly coupled to the length of the nascent chain (Josefsson & Randall, 1981). It is therefore interesting that, unlike the behaviour of the signal region of ovalbumin, Gierasch and her colleagues have observed that synthetic signal sequences from the receptor protein of *Escherichia coli* λ phage penetrate phospholipid monolayers (Briggs *et al.*, 1985). (It remains possible, however, that the extremely low ionic strength used by these workers may have had significant effects on the surface properties of the peptides studied.)

Secondly, it was proposed by Fidelio *et al.* (1986*c*) that the insertion of a completed polypeptide chain into the endoplasmic reticulum membrane and the loss of its signal peptide by proteolytic cleavage could, as a consequence of the lower stabilities of mature proteins at high surface pressures, result in the mature protein being extruded into the cisternae, i.e. in protein translocation. This suggestion was supported by the fact that, like the putative signal sequence of ovalbumin, the pretrypsinogen 2 signal sequence and a synthetic 'consensus' signal sequence also have higher surface stabilities than polypeptides and proteins (including melittin and secreted proteins) that have been previously investigated (Fidelio *et al.*, 1986*a*). The observations reported here provide further support for our previous proposal because it has now been found that the ovalbumin signal peptide can withstand a higher lateral surface pressure than mature ovalbumin in mixed films with phospholipids, as well as in the single component monolayers that were investigated previously.

Since the behaviour towards phospholipid monolayers of the isolated tryptic fragment, bearing the signal region of ovalbumin, is clearly distinct from that of mature ovalbumin, it would seem that the signal region (which is present in the mature protein) makes only a limited contribution, if any, to the surface properties of mature ovalbumin. Moreover, the isolated signal region has been shown to be active in translation systems *in vitro* in competing for the signal in nascent preprolactin, leading to inhibition of processing (Robinson *et al.*, 1986). Presumably, the signal region in mature ovalbumin is involved in tertiary folding and disulphide-pairing, and is thus buried and hidden from the surface of the protein, whereas the environment of the signal in a nascent chain that is emerging from the ribosome may be more akin to that in the isolated tryptic fragment. In keeping with this conclusion is the finding that ovalbumin has similar surface properties to other soluble proteins, e.g. α-lactalbumin and lysozyme, which do not contain uncleaved signal sequences.

In the light of our observations we envisage that, following release of the ovalbumin signal peptide from its putative receptor into the interior of the membrane, the signal peptide will be completely miscible with membrane phospholipids, as it is unlikely that the molar concentration of peptide will achieve the concentration required (approx. > 0.2) for two immiscible phases to be formed. Even if a separate peptide-rich phase is formed, the peptide will probably remain in the membrane, since our data on the behaviour of mixed monolayers of the signal peptide with phospholipids (Figs. 7*a* and 7*c*) indicate that the collapse pressure of any peptide-rich phase will probably exceed 30 $mN \cdot m^{-1}$. It seems probable that the bulk of the newly synthesized ovalbumin will then enter the membrane spontaneously, in view of the fact that mature ovalbumin can penetrate phospholipid monolayers from the subphase, apparently independently of the type of phospholipid present. Once this occurs, the critical molar concentration of ovalbumin for the separation of protein-rich and lipid-rich phases in the membrane, which on the basis of our experiments will be very low and of the order of 0.0025–0.005, will quickly be exceeded. It is anticipated that this will result in the mature ovalbumin molecule being extruded from the membrane since, at high molar ratios of ovalbumin in mixed protein/phospholipid monolayers, the collapse pressure of the separate protein-rich phase is only some 22–24 $mN \cdot m^{-1}$ (Figs. 6*a* and 6*c*).

Our monolayer experiments do not indicate why proteins are translocated across membranes rather than merely being extruded from the side on which they entered. This is presumably a feature of the asymmetric nature of biological membranes and of their immediate cellular environment. Our proposals do, however, offer a possible basis for explaining the translocation of ovalbumin across endoplasmic reticulum membranes, and perhaps also the movement of other proteins across membranes, in terms of the physical properties of membrane phospholipids, signal sequences and mature proteins, rather than in terms of the behaviour of as yet uncharacterized entities, such as proteinaceous, water-filled channels or some 'changed environment' that causes the interior of the bilayer to be more amphiphilic, through which proteins move (Walter *et al.*, 1984). The dynamic model that is suggested here, which attempts to take account of likely interactions between membrane phospholipids and the polypeptide entities concerned, also has the advantage [by comparison with the spontaneous insertion and membrane trigger models of protein translocation proposed by von Heijne & Blomberg (1979), Wickner (1980) and Engelman & Steitz (1981)] that it offers a possible mechanism for both the entry into and the exit of the protein from the membrane.

It has recently been commented by von Heijne (1985) that none of the models that has been suggested to date comes out clearly ahead of its competitors. According to him, the loop, helical hairpin, and membrane trigger hypotheses have all been discredited as generally valid

models by experimental data, the direct transfer model has not been able to live up to all of its predictive aspirations, and the signal hypothesis leaves so many important questions unanswered regarding the actual workings of the export machinery that it is more of a research paradigm than a specific, quantifiable model. While this may be an extreme view, we suggest that further attention to the possible involvement of lipid–protein interactions and of the relative lateral stabilities of signal sequences and mature proteins in the movement of proteins across biological membranes may assist understanding of the translocation process.

This work was supported by a grant from the Wellcome Trust.

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Received 14 October 1986/7 January 1987; accepted 6 February 1987