Properties of signal-sequence peptides at an air–water interface

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The surface behaviour of three signal-sequence polypeptides (the pretrypsinogen 2 signal sequence, a synthetic consensus signal sequence and the putative signal sequence of ovalbumin) were studied at an air–water interface. It was found that the surface stabilities of the spread polypeptide films were higher than those of polypeptides and proteins previously investigated (including melittin and membrane proteins), and that the signal peptides had a much lower affinity for the interface than had other peptides and proteins. The observed molecular areas of the signal-sequence peptides indicated that the molecules have a considerable degree of secondary structure at the surface interface.

INTRODUCTION

Although the need for a signal sequence of about 15–30 residues long to assist the translocation of proteins across membranes is generally accepted (Austen & Ridd, 1981), two different models for this process have been proposed. According to the signal hypothesis, a proteinaceous system is required in the membrane to translocate the nascent protein chain synchronously with translation (Blobel & Dobberstein, 1975; Walter & Blobel, 1980; Meyer *et al.*, 1982), whereas the alternative model suggests that the insertion into membranes is thermodynamically driven by the conformational properties of the nascent protein and by protein–membrane lipid interactions (von Heijne & Blomberg, 1979; Wickner, 1980; Engelman & Steitz, 1981). The properties and conformation of the signal peptide would seem to be critical for the translocation process, however, whichever mechanism is correct (Austen & Ridd, 1981). In the present work we have therefore investigated the behaviour of three signal-sequence peptides at an air–water interface.

MATERIALS AND METHODS

The monolayer equipment used was similar to that described previously (Maggio & Lucy, 1975; Fidelio *et al.*, 1981). The pretrypsinogen and consensus signal peptides were made on solid-phase supports (Austen & Ridd, 1981; Austen *et al.*, 1984); the ovalbumin signal peptide was isolated from a tryptic digest of ovalbumin and it was used in the *S*-sulphonylated ($S\text{-SO}_3^-$ form) (Robinson *et al.*, 1986). The M_r values of these peptides were as follows: pretrypsinogen peptide, 1667; consensus peptide, 2066; ovalbumin peptide, 3057. All experiments were carried out at $21 \pm 1^\circ\text{C}$ on a subphase of unbuffered 145 mM-NaCl, and the pH values specified were obtained by adjusting the subphase with HCl (2 M) or NaOH (2 M). To obtain surface pressure–surface area isotherms, the consensus and ovalbumin peptides were spread at the interface either from an aqueous solution or from a suitable solvent system [chloroform/methanol/dimethyl sulphoxide/ethanol/water (2:5:2:3:2, by vol.) for the consensus peptide, and chloroform/methanol/

dimethyl sulphoxide (3:2:2, by vol.) for the ovalbumin peptide]. The pretrypsinogen peptide was insoluble in water at high concentrations, and it was therefore dissolved in pure dimethyl sulphoxide or in chloroform/methanol/dimethyl sulphoxide (3:2:2, by vol.). The water used was double-distilled in an all-glass apparatus. All solvents were AristaR grade from BDH Chemicals, Poole, Dorset, U.K. NaCl (AnalaR grade from BDH Chemicals) was heated at 400°C for 6 h before use. Dioleoyl phosphatidylcholine was from Sigma Chemical Co., Poole, Dorset, U.K. The absence of surface-active impurities from the solvents and from the subphase was demonstrated by the small changes in surface potential ($< 5\text{ mV}$) and surface pressure ($< 0.1\text{ mN}\cdot\text{m}^{-1}$) that occurred when a clean interface was compressed. For surface pressure–area experiments, the area of the Teflon trough was 92 cm^2 , and the rate of compression was $6.4\text{ cm}^2\cdot\text{min}^{-1}$. For adsorption experiments, the area of the trough was decreased to 16.1 cm^2 , and the samples were injected into the subphase in small volumes (maximum $20\text{ }\mu\text{l}$) from aqueous solution (consensus and ovalbumin peptides) or from dimethyl sulphoxide (pretrypsinogen peptide). After injections, the subphase was stirred for 0.5 min with a magnetic stirrer.

All experiments were done at least in triplicate; they were reproducible within $\pm 1\text{ mN}\cdot\text{m}^{-1}$ for surface pressure and within $\pm 5\%$ for surface potential and molecular areas.

RESULTS AND DISCUSSION

Two of the signal peptides studied, the synthetic pretrypsinogen 2 peptide and the synthetic consensus peptide (which constitutes a consensus of known signal sequences), have previously been shown to compete effectively with naturally occurring signal sequences of several preproteins, thus inhibiting the processing *in vitro* of preproteins (Austen & Ridd, 1981; Austen *et al.*, 1984). The other peptide used was the putative signal sequence that is contained within ovalbumin (Tabe *et al.*, 1984). This peptide has been isolated as a tryptic fragment from ovalbumin and shown to inhibit completely the processing of preprolactin (Robinson *et al.*, 1986). The primary structures of these peptides are

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Pretrypsinogen signal sequence

Ala-Lys-Leu-Phe-Leu-Phe-Leu-Ala-Leu-Leu-Leu-Ala-Tyr-Val-Ala

Consensus signal sequence

Lys-Lys-Ser-Ala-Leu-Leu-Ala-Leu-Met-Tyr-Val-Cys-Pro-Gly-Lys-Ala-Asp-Lys-Glu

Putative signal sequence of ovalbumin

²¹**Val-His-His-Ala-Asn-Glu-Asn-Ile-Phe-Tyr-Cys-Pro-Ile-Ala-Ile-Met-Ser-Ala-Leu-Ala-Met-Val-Tyr-Leu-Gly-Ala-Lys**⁴⁷

Melittin (Habermann, 1972)

Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Gln-Gln

Fig. 1. Primary structures of the pretrypsinogen, consensus and ovalbumin signal-sequence peptides, and of melittin

shown in Fig. 1. The consensus and ovalbumin peptides form insoluble surface monolayers exhibiting properties that are independent of whether the films have been spread from an aqueous solution or from an organic solvent, indicating that the organizations of the peptides at the interface are independent of their conformation in the spreading solvent.

The surface pressure-surface area isotherms obtained for the signal-sequence peptides are shown in Fig. 2. Since the characteristics of these isotherms were unchanged and the decrease in molecular area was less than 5% after two successive cycles of compression and expansion, the peptides appear to form true insoluble monolayers. The

values of surface compressibility at $20 \text{ mN} \cdot \text{m}^{-1}$ were between 1.6×10^{-2} and $2.2 \times 10^{-2} \text{ m} \cdot \text{mN}^{-1}$, and were similar to those found for liquid-expanded glycosphingolipids and phospholipids (Maggio *et al.*, 1978; Fidelio *et al.*, 1982, 1986). This indicates that the peptides at the interface are effectively in a liquid state. The surface potentials for the three peptides were found to lie within the range 200–250 mV, indicating that, in spite of their differing sequences, the overall, long-range, dipolar organizations of the peptides at the interface are similar.

The most interesting features of the surface behaviour of the signal-sequence peptides are the values obtained for their collapse pressures. The collapse pressure of a monolayer is a measure of its stability, and it represents the maximum lateral pressure that can be sustained before a monomolecular anisotropic film disrupts and gives rise to bulk material at the interface. As shown in Fig. 2, the three signal peptides exhibited well-defined collapse pressures, with values ranging from 24 to 43 $\text{mN} \cdot \text{m}^{-1}$. These values are greater than the collapse pressure (21 $\text{mN} \cdot \text{m}^{-1}$) of the highly amphipathic peptide melittin (Fidelio *et al.*, 1984), which resembles signal sequences in that it contains a high proportion of hydrophobic amino acids (Fig. 1). The collapse pressures of the three signal peptides are also greater than the reported values for nine other proteins that are listed in the legend to Fig. 2.

At surface pressures that are relevant to biological membranes (20–30 $\text{mN} \cdot \text{m}^{-1}$; cf. van Deenen *et al.*, 1976; Israelachvili *et al.*, 1980), the pretrypsinogen peptide has a molecular area of 0.7 nm^2 . This value is consistent with an extended β -structure for the peptide, orientated perpendicular to the interface with both its *N*-terminal alanine and basic lysine residue in the subphase, assuming an average length of amino acid side chains of about 0.5 nm (cf. Smith *et al.*, 1983). Such an orientation is supported by the effect of high pH on the surface potential per molecule of this peptide (Fig. 3), since a change in pH will only affect the surface potential in the way observed if the *N*-terminus, and possibly the basic lysine residue also, are in the aqueous subphase. [The surface pressure-area isotherm for the pretrypsinogen peptide was unaffected by pH (results not shown).] By contrast, the observed minimum molecular areas for the consensus and ovalbumin peptides (about 1.6 nm^2) are consistent with an α -helical structure perpendicular to the interface or, alternatively, with a 'loop' structure in which two antiparallel β -strands are linked by a β -turn

Fig. 2. Surface isotherms of the signal-sequence peptides

Surface pressures (—) and surface potentials (----) for the pretrypsinogen signal-sequence peptide (●), the consensus signal-sequence peptide (○) and the putative ovalbumin signal-sequence peptide (■) are shown. The subphase was 145 mM-NaCl at pH 7. The upper arrow on the ordinate indicates the value of the collapse pressure of dioleoyl phosphatidylcholine. The lower arrow indicates the average collapse pressure of the following proteins: melittin, 21.2 $\text{mN} \cdot \text{m}^{-1}$; myelin basic protein, 14.2 $\text{mN} \cdot \text{m}^{-1}$; bovine serum albumin, 13.1 $\text{mN} \cdot \text{m}^{-1}$ (Fidelio *et al.*, 1984); α -lactoglobulin, 18 $\text{mN} \cdot \text{m}^{-1}$; α -lactoalbumin, 20 $\text{mN} \cdot \text{m}^{-1}$ (Mitchell *et al.*, 1970); cytochrome *c*, 13 $\text{mN} \cdot \text{m}^{-1}$ (Quinn & Dawson, 1969); Folch-Lees proteolipid, 21 $\text{mN} \cdot \text{m}^{-1}$ (Colacicco, 1972); β -casein, 23 $\text{mN} \cdot \text{m}^{-1}$; total high-density apolipoprotein, 20 $\text{mN} \cdot \text{m}^{-1}$ (Phillips *et al.*, 1975); lysozyme, 25 $\text{mN} \cdot \text{m}^{-1}$ (Adams *et al.*, 1971).

Fig. 3. Effect of pH on the surface potential of the pretrypsinogen signal-sequence peptide

Values of surface potential per molecule of the pretrypsinogen peptide are shown for the pH values indicated. The subphase was 145 mM-NaCl.

region (Austen *et al.*, 1984). [This also assumes an inner diameter of 0.5 nm for the α -helix, and a distance between the two opposite β -strands of 0.4 nm (cf. Smith *et al.*, 1983).]

The adsorption behaviour (from the subphase to the interface) for the consensus peptide is shown in Figs.

4(a)–4(c). Equilibrium was achieved very slowly when this peptide was injected beneath a clean interface (Fig. 4a), and the pressure observed was dependent on the concentration of the peptide in the subphase (Fig. 4b). For proteins that reach equilibrium very slowly, the equilibrium pressure has been defined as the maximum surface pressure achieved when the rate of change of pressure is less than $0.05 \text{ mN} \cdot \text{m}^{-1} \cdot \text{min}^{-1}$ (Fidelio *et al.*, 1982). The maximum equilibrium surface pressure for the consensus peptide was thus approx. $11 \text{ mN} \cdot \text{m}^{-1}$ at a peptide concentration of $0.8 \mu\text{M}$ (Fig. 4b). For the pretrypsinogen peptide, a corresponding value of $10 \text{ mN} \cdot \text{m}^{-1}$ was obtained at a peptide concentration of $4 \mu\text{M}$. [At this concentration the peptide is soluble in aqueous solution (cf. Austen & Ridd, 1981).] The subphase concentrations of these signal peptides that were required to reach a maximum value of surface pressure were thus much higher than those for melittin ($0.18 \mu\text{M}$), for the membrane protein glycophorin ($0.2 \mu\text{M}$), or even for a soluble protein, such as albumin ($0.2 \mu\text{M}$) (Fidelio *et al.*, 1982). The ovalbumin peptide was unable to partition to a clean interface at a concentration of $1 \mu\text{M}$ (the maximum concentration that was tested).

The surface potentials developed at the interface on adsorption of the signal peptides from the bulk phase are similar to those obtained, at comparable surface pressures, for the corresponding films made by spreading. [For the consensus peptide, compare Fig. 2 with Fig. 4(c).] This implies that the dipolar organizations of the

Fig. 4. Adsorption behaviour of the consensus signal-sequence peptide

(a) Time course for surface pressure changes following injection into the subphase of the peptide at a concentration of 0.4 mM. (b) and (c) The surface pressure and surface potentials respectively observed for the concentration of peptide indicated. The subphase was 145 mM-NaCl at pH 7.

peptides at the interface are independent of the way in which the monolayers are formed.

It is apparent from these experiments that the ability of signal peptides to adsorb from the subphase on a clean air-water interface is less than is observed for melittin and for serum albumin (Fidelio *et al.*, 1982). This suggests that for signal peptides to penetrate into membranes *in vivo* they need either to be protected from the aqueous intracellular environment (perhaps by remaining in the ribosomal cleft until the ribosome becomes attached to the membrane by the signal recognition particle), and/or to be introduced into the interior of the membrane via interactions with a receptor protein in the membrane.

The pressure-area isotherms of the signal-sequence peptides in Fig. 2 indicate that signal sequences have a greater surface stability and can support a much higher lateral pressure, after incorporation into a membrane, than all proteins and peptides that have been previously investigated, including the amphipathic peptide melittin. The limiting molecular areas of melittin, the consensus peptide and the ovalbumin peptide are similar (1.80, 1.58 and 1.50 nm² respectively), and each peptide may be α -helical in structure at the air-water interface. The monolayers of signal peptides, however, have collapse pressure that are more like those of phospholipids than those of melittin and of other proteins. Indeed, the collapse pressure of the consensus signal peptide (43 mN·m⁻¹) is similar to that of dioleoyl phosphatidylcholine (Fig. 2). This may be of significance for the translocation of signal peptides across membranes.

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