Isolation and properties of the signal region from ovalbumin

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A tryptic fragment (residues 21–47) containing the signal peptide of ovalbumin has been isolated by reverse-phase chromatography. The peptide is more active at inhibiting the processing of pre-prolactin in an in vitro translation system than fragments of ovalbumin isolated previously, and is similar in properties to synthetic signal peptides. The ovalbumin signal fragment is shown to bind to a protein component of salt-stripped pancreatic microsomal membranes, which is cross-linked under UV irradiation by a radioactive synthetic photoaffinity signal peptide probe to yield a radiolabelled 45 kDa protein.

Signal sequence *Ovalbumin* *Translation* *Protein translocation* *Signal receptor*

1. INTRODUCTION

Secretory proteins are generally formed by cleavage of the amino terminal extensions of precursors in the lumen of the endoplasmic reticulum (ER) [1]. These extension sequences, called signal peptides, are thought to assist transfer of the proteins through the membrane of the ER from their sites of synthesis in the cytoplasm by interacting with a signal recognition particle [2]. Chicken ovalbumin is an exception in that its signal peptide is not cleaved [3]. Lingappa et al. [4] isolated a tryptic fragment from ovalbumin comprising residues 229–276, which at concentrations between 0.45 mg/ml and 0.9 mg/ml, inhibited processing of nascent pre-prolactin by rough microsomes during translation in vitro, but in contrast to these studies, examination of the properties of nascent ovalbumin chains in in vitro translation systems located a functional signal within the N-terminal 70 residues [5]. Studies defining hybrid proteins, consisting of segments from the N-terminal region of ovalbumin fused to α-globin, that are sequestered by oocyte membranes showed that the signal of ovalbumin is located between residue 22 and 41 [6]. Here we describe the isolation of a fragment of ovalbumin containing the signal region, and examine its interactions with membranes from the ER.

2. MATERIALS AND METHODS

Recrystallized ovalbumin (Sigma, UK) (225 mg) in 6 M guanidine HCl, 0.1 M Tris-HCl (pH 7.4) (20 ml) was treated with three successive cycles of sodium sulphite (158 mg) (30 min) followed by sodium tetrathionate (385 mg) (10 min). After dialysis, the modified protein in 0.05 M ammonium bicarbonate, 0.5 mM $CaCl_2$ was digested with TPCK-trypsin (6 mg) for 3 h at 37°C, then PMSF (2 mg) was added. The digest was fractionated by repeated injection (1.2 ml) on Aquapore RP-300 (0.7 × 25 cm) (Brownlee Labs, CA, USA) and eluted with an acetonitrile gradient from 35 to 50% in 0.1% TFA.

S-sulphonylated peptides and nuclease-stripped pancreatic rough microsomes (A_{260} 72) were pre-incubated with dithiothreitol (1.4 mM) for 10 min at 37°C to remove the *S*-sulphonyl group [7] then added to a wheat germ translation system programmed with bovine pituitary mRNA and [^{35}S]methionine, and the extent of processing was

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monitored [8]. Photoreaction was performed by incubating salt-stripped microsomes (700 μg protein) with ^{125}I-labelled photoreactive *S*-azidophenacyl-^{12}Cys signal peptide [9] (200 nM) under UV irradiation for 2 h at 4°C from an SL-58 multiband UV light (Ultraviolet Products, San Gabriel, CA), together with the ovalbumin peptide either *S*-sulphonylated or reduced.

3. RESULTS

The most retained fragment from trypsin-digested ovalbumin eluting after 20 min from a reverse-phase HPLC column (fig.1) was found to be homogeneous by rechromatography on HPLC, Sephadex G-50 in 30% acetic acid, and dansyl determination [10], which showed that Val was the only N-terminal residue. Comparison of the amino acid analysis (Asp, 2.11; Ser, 0.96; Glu, 1.17; Pro, 1.20; Gly, 1.16; Ala, 4.83; Val, 2.07; Met, 2.00; Ile, 3.05; Leu, 2.19; Tyr, 1.82; Phe, 1.11; His, 1.93; Lys, 1.07) to the published sequence of ovalbumin [11] identified the fragment as residues 21–47, the sequence of which is 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. Reduced in the presence of nuclease-stripped rough microsomal membranes, the peptide prevented microsome-catalysed conversion of nascent ^{35}S-preprolactin to ^{35}S-prolactin progressively up to 25 μM (tracks 1–7, fig.2), whereas pooled peptides derived from the rest of ovalbumin obtained from preceding functions on HPLC (fig.1) inhibited translation at 25 μM (1 mg/ml), but did not prevent processing to ^{35}S-prolactin (track 10, fig.2).

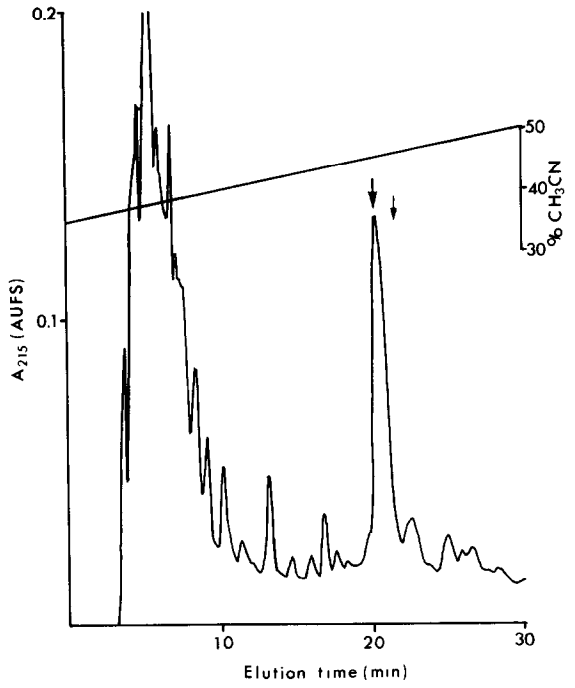

Fig.1. HPLC profile of tryptic fragments from *S*-sulphonylated ovalbumin. The elution position of the *S*-sulphonylated signal fragment (res. 21–47) is shown with a large arrow, and the elution position of the peptide after reduction and re-chromatography is shown with a small arrow.

Interaction of the ovalbumin signal peptide with a component of salt-stripped microsomal membranes that binds signal peptides was assessed by inhibition of the formation of a covalent linkage to that component by a radioactive photoreactive signal peptide probe. In the absence of competing peptides, the ^{125}I-azidophenacyl derivative of a synthetic signal peptide irradiated in the presence of microsomal membranes links to produce a ^{125}I-labelled 45 kDa protein adduct [9]. The autoradiograph of microsomal membrane proteins separated by SDS-PAGE (fig.3) showed that the ovalbumin peptide effectively inhibited the production of this 45 kDa labelled protein, but the production of other minor labelled components, which presumably results from non-specific binding, was not affected. The ovalbumin peptide

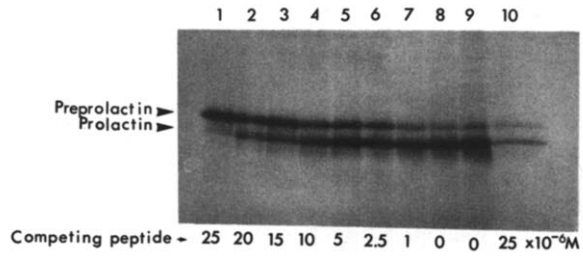

Fig.2. SDS-PAGE (15% acrylamide) of ^{35}S-labelled proteins translated in a wheat germ lysate from bovine pituitary mRNA with pancreatic rough microsomes at a final concentration of 7.2 A_{260} together with the ovalbumin signal fragment (res. 21–47) (tracks 1–7), or peptides from the rest of ovalbumin (track 10).

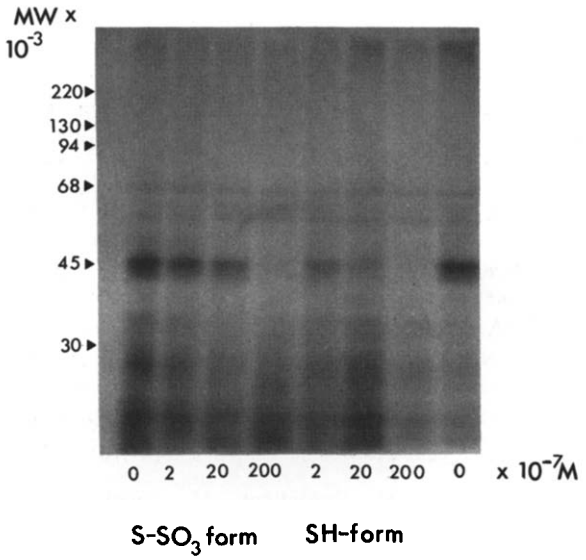

Fig.3. SDS-PAGE (7–16% gradients of acrylamide) of ^{125}I-labelled proteins in salt-stripped pancreatic microsomes resulting from photoreaction with ^{125}I-azidophenacyl-signal peptide, together with the ovalbumin signal fragment (res. 21–47).

was inhibitory both in the reduced and, more weakly, in the *S*-sulphonylated form.

4. DISCUSSION

We have developed a one-step chromatographic method for the isolation of a tryptic region from ovalbumin previously shown by genetic manipulation and production of fused proteins [6] to contain the internal signal sequence. The extreme hydrophobicity of this peptide leads to its greater retention on reverse-phase chromatography than any other fragment.

After reduction to its native form, the peptide is retained a further 1.4 min on HPLC (fig.1), but becomes more difficult to isolate owing to its decreased solubility. The isolated signal fragment blocks the microsome-catalysed conversion of preprolactin to prolactin at 25 μM (75 μg/ml), and is thus about 13-fold more inhibitory than a fragment consisting of a central portion of ovalbumin under similar conditions of in vitro translation [4]. It acts at similar concentrations to synthetic signal peptides resembling the signal regions of pre-pro-PTH [12], pre-trypsinogen [13], MOPC-321 immunoglobulin light-chain precursor [14], and a consensus of known signals [8], by preventing access of nascent preprolactin to signal peptidase on the lumenal side of microsomal vesicles [8,12]. The concentrations at which signal peptides inhibit processing are high in relation to the estimated amount of ^{35}S-preprolactin processed in one round of translation (0.5 pmol/ml) but low in relation to the amount of microsomal protein required for processing (about 1 mg/ml). Thus, the isolated signals are relatively inefficient in comparison to signals present in nascent chains attached to polysomes, presumably because the ribosome has an important function in targetting to the ER membrane [2]. The in vitro processing system itself is also relatively inefficient.

Microsomal membranes contain a number of proteins involved in binding polysomes containing mRNA for secretory proteins [2] or binding the nascent chain to the membrane [12]. A photoreactive signal peptide has recently been shown to covalently link to an integral membrane protein in rough microsomes yielding an adduct of 45 kDa; the specificity of binding that gives rise to this adduct has been shown by inhibition of labelling by signal peptides, but not by control hydrophobic peptides [9]. The ovalbumin fragment (residues 21–47) also binds, and displaces binding to the photoreactive probe. Although it is not clear what role the protein that binds the signal peptide plays, it could have a role in translocation, or degradation of the signal peptide, and its occurrence in membranes from tissues active in processing secretory protein [9], and the inhibition of its cross-linking by the natural signal from ovalbumin lends credence to the view that the microsomal protein is indeed functional in protein secretion.

In most signal peptides, the length of the hydrophobic region varies from 7 to 20 amino acids, with most signals having between 10 and 15 hydrophobic residues [16,17]. A hydropathy plot (9 residue span) [18] shows that the signal region of ovalbumin contains a stretch of 17 residues in length (residues 28–44) of almost equal hydrophobicity to that in the cleavable signal of pre-somatotropin, and from the maximum hydrophobicity (19.3) calculated from residues 33–40 [19], and net positive charge of the sequence on its immediate N-terminal side (Lys-Val-His-His-Ala-Asn-Glu; residues 20–26), it would fulfill predictive criteria [19] as a signal region.

The reason for the lack of cleavage of the ovalbumin signal by signal peptidase in the ER lumen during secretion in vivo is not clear. The primary or secondary structure present on the C-terminal side of the signal may not satisfy the specificity requirements of signal peptidase [13,20,21]. Alternatively, the unusual internal location of the signal over 20 residues from the amino terminus of ovalbumin, may prevent access to signal peptidase, although a signal sequence internally transposed by genetic manipulation has previously been shown to retain its function, and is cleaved [23]. In the case of ovalbumin, however, the amino terminal 20 residue section may have an unusual function in allowing the uncleaved signal to move from the membrane into the lumen of the ER [6].

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