

Identical Precursors for Serum Transferrin and Egg White Conalbumin*

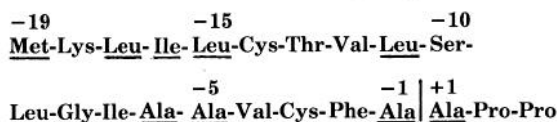
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SUMMARY

The NH₂-terminal sequences of egg white conalbumin and chicken serum transferrin were examined and found to be identical. Conalbumin, when synthesized in a rabbit reticulocyte cell-free translation system, was found to contain an NH₂-terminal extension of 19 amino acid residues. Sequential Edman degradation of this precursor (pre-conalbumin) labeled with radioactive amino acids revealed the following sequence:



The vertical line indicates the site at which pre-conalbumin is cleaved to yield authentic conalbumin. The sequence represents the primary translation product since the NH₂-terminal methionine was shown to be derived from initiator Met-tRNA^{Met}_f.

A partial NH_2 -terminal sequence of transferrin synthesized *in vitro* was also determined (underlined residues) and it is identical with that of pre-conalbumin.

The mechanisms by which polysomes directing the synthesis of secretory proteins associate with the endoplasmic reticulum and subsequently pass their translation products into the lumen are poorly understood. It has been postulated by Blobel and his co-workers (1) that secretory proteins are initially synthesized containing an NH₂-terminal extension composed primarily of hydrophobic residues. The hydrophobic nature of this transient leader sequence serves as a signal which allows membrane-bound receptors to recognize and anchor ribosomes that are in the process of translating secretory proteins. The signal peptide, after serving its presumed function, is removed before the nascent chain is completed. By translating the mRNAs coding for secretory proteins in cell-free systems, which apparently lack the processing enzyme, it is possible to demonstrate the presence of NH₂-terminal leader sequences on the synthesized products. Sequence analyses of these extensions (typically 15 to 30 residues long) has now been presented for a number of secretory proteins (2-9).

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The chicken oviduct presents a unique system in which a variety of secretory proteins originating from the same tubular gland cell (10) can be examined for the presence of signal peptides and any possible sequence homology that may exist among them. Such a comparison would be useful in understanding their properties and function(s). The oviduct of laying hens is a major secretory organ with ovalbumin, conalbumin, ovomucoid, and lysozyme constituting approximately 80% of its total synthetic effort. Previous investigations have shown that both lysozyme (11) and ovomucoid¹ are synthesized *in vitro* as precursors, whereas ovalbumin (12) is not. This study provides evidence that conalbumin, when synthesized in a reticulocyte lysate, contains an NH₂-terminal extension of 19 amino acids and that its sequence is not homologous to either that of pre-ovomucoid or pre-lysozyme.

In addition, we have examined the NH₂-terminal sequence of transferrin synthesized *in vitro* and have compared it to that of pre-conalbumin. Transferrin is the major serum iron-binding protein which is synthesized primarily by liver hepatocytes (13). Accumulating evidence suggests that conalbumin (ovotransferrin) and transferrin are products of the same gene, differing only in carbohydrate content (14). Both proteins have a molecular weight of approximately 77,000 and have been shown to be identical polypeptides by a number of criteria including immunological cross-reactivity, amino acid composition, peptide maps, and iron-binding properties (15, 16). Recently, the mRNAs coding for conalbumin and transferrin were shown to be identical by size and hybridization properties (17). This investigation demonstrates that both conalbumin and transferrin have the same NH₂-terminal sequence, and when synthesized *in vitro*, contain identical NH₂-terminal leader sequences.

EXPERIMENTAL PROCEDURES

Materials—Tritiated amino acids were purchased from New England Nuclear, Schwarz/Mann, or Amersham/Searle at the highest specific activities available. [^{35}S]Methionine and [^{35}S]cysteine were prepared from bacteria grown on $\text{H}_2^{35}\text{SO}_4$ (18). Omnifluor and Aquasol are products of New England Nuclear.

Protein Preparation.—Conalbumin was extracted from egg white and purified by DEAE-cellulose chromatography essentially as described (19). The NH_2 -terminal cyanogen bromide fragment of conalbumin was purified by loading a CNBr digest (20) onto a Bio-Gel P-100 column (1.5×80 cm) equilibrated with 10% acetic acid at a flow rate of 20 ml/h. The largest peptide was collected and lyophilized (21).

Serum transferrin was first enriched by loading chicken serum onto an anti-conalbumin Sepharose column (2.5 × 25 cm) equilibrated with 15 mM sodium phosphate, 150 mM NaCl, pH 7.1. The column was washed with this buffer followed by several column volumes of buffer containing 1.4 M NaCl and the adsorbed transferrin was eluted with 0.1 M glycine, pH 2.8. The fractions containing protein were pooled, dialyzed *versus* distilled H₂O, lyophilized, and further purified by DEAE-cellulose chromatography as described for conalbumin.

Anti-conalbumin was obtained from goats immunized with conalbumin mixed 1:1 with Freund's complete adjuvant as described (22). Goat anti-conalbumin immunoglobulins were partially purified by ammonium sulfate (40%) precipitation and coupled to Sepharose CL-6B according to the procedure of Cuatrecasas (23).

Preparation of mRNA and tRNA—RNA was isolated from laying hen oviduct or liver by homogenizing the tissue in 1% sodium dodecyl sulfate containing 50 $\mu\text{g}/\text{ml}$ of proteinase K, followed by phenol/chloroform extraction. Messenger RNA was enriched on an oligo(dT)-

¹ S. N. Thibodeau, R. D. Palmiter, and K. A. Walsh, manuscript in preparation.

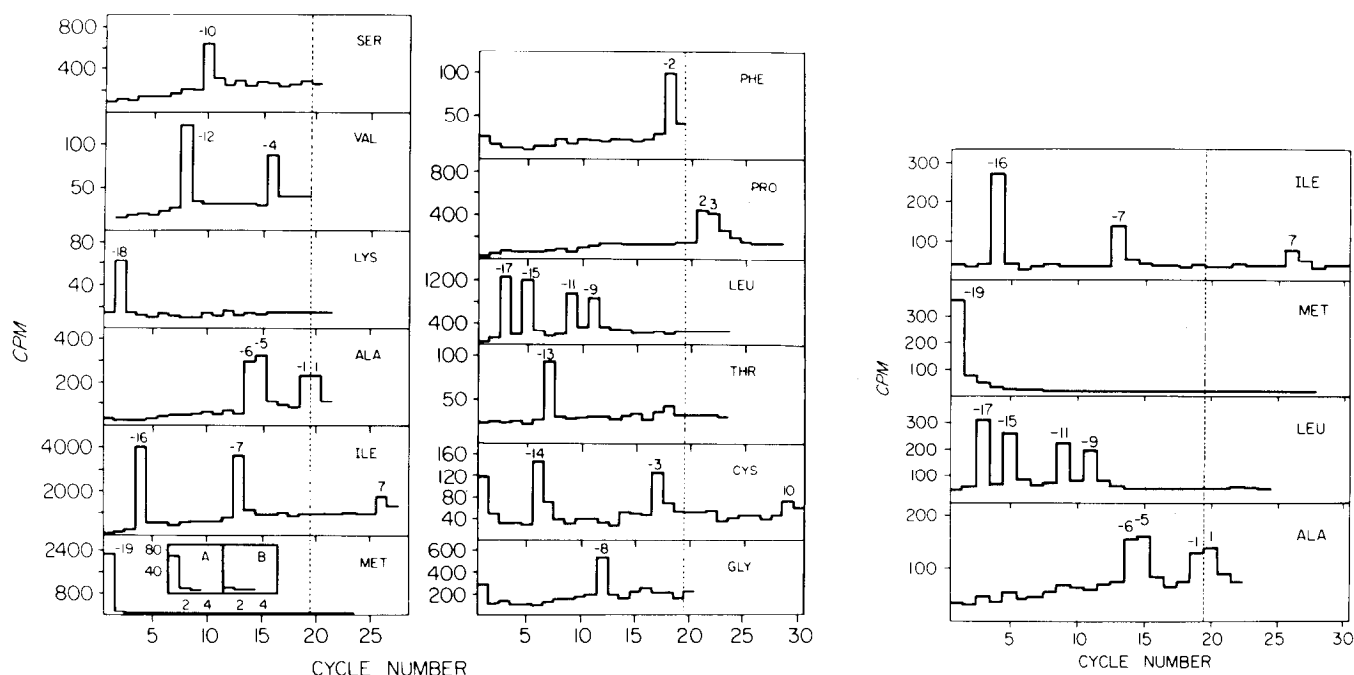


FIG. 1 (left). Automated Edman degradation of pre-conalbumin labeled with a mixture of radioactive amino acids. All amino acids were tritiated except [^{35}S]cysteine and [^{35}S]methionine. The data shown for [^3H]alanine, [^3H]valine, [^3H]glycine, [^3H]lysine, and [^3H]phenylalanine were from multilabeled experiments. Pre-conalbumin labeled with the remaining amino acids were sequenced separately in

single- or double-labeled experiments. Sequence analyses of pre-conalbumin labeled with [^{35}S]methionine from Met-tRNA $^{\text{Met}}$ and Met-tRNA $^{\text{Met}}$ are shown in insets A and B, respectively.

FIG. 2 (right). Automated Edman degradation of pre-transferrin labeled with a mixture of radioactive amino acids. The data for each amino acid are the result of separate sequence experiments.

quence data from four separate experiments in which the translation product of transferrin mRNA was examined. It is clear that transferrin is also synthesized as a precursor, pre-transferrin. The NH $_2$ -terminal extension is 19 residues long and the amino acids in all 10 positions determined by these experiments are identical with those of pre-conalbumin (Table I).

DISCUSSION

We examined the NH $_2$ -terminal sequence of conalbumin synthesized in a membrane-free translation system to ascertain whether this secretory protein is synthesized as a precursor with a hydrophobic leader sequence as is the case with most secretory proteins. Conalbumin provides a particularly interesting case for analysis since its leader sequence can be compared with that of transferrin, an apparently identical protein synthesized in the liver, as well as with those of other egg white proteins. These comparisons may help elucidate the role of these leader sequences in protein secretion.

Comparison of the NH $_2$ -terminal sequence of conalbumin synthesized *in vitro* with that of secreted conalbumin shows that the primary translation product from conalbumin mRNA contains a 19-amino-acid NH $_2$ -terminal extension (Table I). Fifteen of these residues are nonpolar and in this respect, it resembles the leader or "signal" sequences reported for other secretory proteins. This precursor is the primary translation product since it begins with methionine which was derived from initiator tRNA. The leader sequence is presumably cleaved from the precursor during membrane transport, but this point has not been directly examined.

A similar analysis of transferrin produced by translation of liver RNA *in vitro* indicates that it too is synthesized as a precursor and that its leader sequence is identical with that of pre-conalbumin. In addition, we have shown that the NH $_2$ -terminal sequence of the mature proteins, conalbumin and transferrin, are indistinguishable. These data corroborate ear-

lier structural and genetic evidence (15, 16) suggesting that both proteins are products of the same gene. If the leader sequences of these two proteins are indeed involved in the secretion mechanism, then the data also indicate that cells of two quite different types recognize the same signal and respond with analogous mechanisms.

The NH $_2$ -terminal sequences obtained for the primary translation products of lysozyme, ovomucoid, and ovalbumin mRNAs are shown in Table I for comparison. Lysozyme and ovomucoid are also synthesized as precursors with hydrophobic leader sequences, but ovalbumin is not. A comparison of the precursor peptides of lysozyme and ovomucoid indicates 6 identities in the sequence of 8 amino acids preceding the cleavage site, but this apparent homology is not shared with conalbumin. The best alignment of the conalbumin leader sequence with either of the other sequences reveals a maximum identity of 4 residues, but such comparisons are tenuous because of the obvious constraint on the amino acids that are selected for these sequences. The amino acid preceding the cleavage site that gives rise to the mature protein is either alanine (pre-conalbumin) or glycine (pre-lysozyme and pre-ovomucoid), suggesting that the protease(s) involved has an elastase-like specificity. We do not yet know, however, if the leader is removed by a single cleavage or whether several intermediates are formed. The removal of the NH $_2$ -terminal methionine in the case of ovomucoid and ovalbumin is presumably unrelated to protein secretion. The finding that 3 secretory proteins produced in the same cell contain different leader sequences suggests that if these sequences are responsible for the vectorial transport of these proteins across the endoplasmic reticulum, then the mechanisms by which they do so are either unique and do not involve a common binding site or else are governed by properties other than the amino acid sequence *per se*. For example, the generally hydrophobic nature of these peptides and/or their secondary and tertiary structures may be important.

Although the NH₂-terminal sequences of the egg white proteins cannot be derived from a common RNA sequence that is joined to the 5'-termini of their various mRNAs, these sequences may have been derived from a common primordial DNA sequence that was joined to these genes and has since diverged into the related sequences that we observe.

The finding that ovalbumin is not synthesized with a transient hydrophobic leader sequence indicates that a major secretory pathway in the oviduct does not require the typical "signal peptide" that is subsequently removed. It may be that special mechanisms have evolved for secretion of ovalbumin, which represents nearly 50% of the total protein synthesized in a hormone-stimulated oviduct, that prevent competition with the other secretory proteins from the same cells. We are currently investigating whether the mechanism of ovalbumin secretion is significantly different than that of the other egg white proteins.

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