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Post-translational cleavage of presecretory proteins with an extract of rough microsomes from dog pancreas containing signal peptidase activity

(deoxycholate extraction/signal peptide/latency)

ROBERT C. JACKSON AND GÜNTER BLOBEL

Department of Cell Biology, The Rockefeller University, New York, New York 10021

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ABSTRACT **The protease(s) responsible for removing the amino-terminal extension of nascent presecretory proteins (signal peptidase) has been extracted from rough microsomes of dog pancreas with the detergent sodium deoxycholate. Preprolactin and pre-growth-hormone, prepared by *in vitro* translation of bovine pituitary RNA in the wheat germ system, were used to assay signal peptidase in the extract. When added to the wheat germ system during translation, the extract reduced the size of preprolactin and pre-growth-hormone to that of prolactin and growth hormone, respectively. Post-translational addition of the extract also reduced the size of preprolactin and pre-growth-hormone to that of the authentic hormones. The prolactin produced by post-translational cleavage of radiolabeled preprolactin has been shown, by partial amino-terminal sequence analysis, to have the correct amino terminus. This post-translational assay has permitted the investigation of the subcellular localization of the enzyme. Sodium deoxycholate extracts of rough microsomes were active, whereas extracts of smooth microsomes were inactive. However, without detergent treatment, neither rough nor smooth microsomes were capable of cleaving preprolactin in the post-translational assay. From this we conclude that the signal peptidase activity is confined to the rough endoplasmic reticulum and is latent. Finally, we have detected two small peptides which we believe could be the signal peptides generated by the endoproteolytic cleavage of preprolactin and pre-growth-hormone by signal peptidase.**

Numerous secretory proteins have been shown to be synthesized as presecretory proteins containing an amino-terminal extension of 15–30 amino acid residues (signal peptide) when their mRNAs are translated in a cell-free system in the absence of microsomal membranes (1–9). In the signal hypothesis (10), it has been proposed that the signal peptide of a nascent presecretory protein nucleates the formation of a functional ribosome–membrane junction which, in turn, provides the topological condition for a cotranslational transfer of the nascent secretory protein across the rough endoplasmic reticulum (RER) membrane into the intracisternal space of the RER. It has been shown for the light chain of immunoglobulin (10, 11) that removal of the signal peptide from the nascent presecretory protein is a cotranslational event—i.e., the signal peptide is removed before the entire secretory protein has been synthesized. Although it has been demonstrated that correct cleavage of nascent presecretory polypeptides can be obtained *in vitro* by adding microsomal membranes cotranslationally to the cell-free system (1, 2, 12), the complexity of the cell-free system has impeded study of the protease (signal peptidase) responsible for removing the signal peptide.

A post-translation assay that is not dependent upon concurrent *in vitro* translation would greatly facilitate the identification, purification, and characterization of the signal peptidase. Post-translational cleavage was not obtained when completed presecretory proteins were incubated with intact microsomal vesicles (1). This suggested that the signal peptidase is present in the microsomal vesicles in a latent form. Previous attempts to disrupt the microsomal vesicles and to obtain post-translational cleavage of immunoglobulin prelight chain were unsuccessful (11).

In this paper we demonstrate that the latency of the microsomal vesicle-associated signal peptidase can be unmasked by detergents and that certain *in vitro* synthesized presecretory proteins such as bovine preprolactin and pre-growth-hormone can be cleaved post-translationally by detergent-solubilized signal peptidase.

METHODS

Preparation of Total Microsomes, Rough Microsomes, and Smooth Microsomes. A postmitochondrial supernatant (10,000 × *g*, 10 min) was prepared from freshly excised dog pancreatic tissue as described (10). This supernatant was divided into two aliquots. One aliquot was centrifuged at 180,000 × *g* for 1 hr, in the A192 rotor of an IEC centrifuge (Damon/IEC Div., Damon Corp., Needham Heights, MA); the pellet of total microsomes was either used immediately or frozen in liquid nitrogen and stored at −80° for future use. Portions (15 ml) of the other aliquot were layered over a discontinuous sucrose gradient comprised of 5 ml of 1.0 M, 5 ml of 1.5 M, 7.5 ml of 1.75 M, and 5 ml of 2.0 M sucrose in 50 mM triethanolamine·HCl, pH 7.5/50 mM KCl/5 mM $MgCl_2$ (Et_3NKM) and centrifuged in the A192 rotor of an IEC centrifuge at 180,000 × *g* for 20 hr. Rough microsomes were collected with a syringe from the 1.75 M sucrose layer and smooth microsomes were collected from the 1.5 M sucrose layer. The membranes were diluted with 1 volume of 0.25 M sucrose-Et_3NKM and layered over a 5 ml cushion comprised of 1.3 M sucrose-Et_3NKM (for rough microsomes) or 1.0 M sucrose-Et_3NKM (for smooth microsomes) and centrifuged at 190,000 × *g* for 1 hr in the A192 rotor. The pellets were used immediately or frozen in liquid nitrogen and stored at −80° for future use.

Preparation of EDTA "Stripped" Membranes. Membrane-bound ribosomes were removed from total or rough microsomes essentially as described by Blobel and Dobberstein (11). Freshly prepared pellets of total or rough microsomes were resuspended by homogenization in 50 mM triethanolamine·HCl (Et_3N) buffer, pH 7.5, to a concentration of 50 A_{280} units/ml [the absorbance of all membrane suspensions was determined

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Abbreviations: RER, rough endoplasmic reticulum; Et_3NKM, 50 mM triethanolamine·HCl, pH 7.5/50 mM KCl/5 mM $MgCl_2$; Et_3N, triethanolamine·HCl; $NaDodSO_4$, sodium dodecyl sulfate.

by dilution into 5% sodium dodecyl sulfate ($NaDodSO_4$)], and an equal volume of an EDTA-containing buffer (50 mM Et_3N, pH 7.5/20 mM EDTA) was added. This suspension was layered over a cushion of 50 mM Et_3N, pH 7.5/50 mM KCl/0.5 M sucrose and centrifuged at 100,000 × *g* for 1 hr. The pellets of stripped membranes were resuspended in 50 mM Et_3N, pH 7.5/50 mM KCl/2 mM $MgCl_2$ at 50 A_{280} units/ml, frozen in liquid nitrogen, and stored at −80° for future use.

Preparation of the Detergent Extract. Fresh or frozen pellets of total microsomes, rough microsomes, or smooth microsomes (see above) were resuspended by homogenization in an appropriate volume of ice-cold buffer (20 mM K *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate, pH 7.6/50 mM NaCl) to a final concentration of 50 A_{280} units/ml. One volume of 10% (wt/wt) sodium deoxycholate was mixed with 19 volumes of the membrane suspension. The clear solution that resulted was centrifuged at 100,000 × *g* for 4 hr. The supernatant was divided into small aliquots, frozen in liquid nitrogen, and stored at −80°. Extract has been stored in this manner for over 6 months without any noticeable loss of activity.

Cell-Free Protein Synthesis. A wheat germ S-23 supernatant was prepared by the method of Roman *et al.* (13). *In vitro* protein synthesis was performed as described (14). A typical 50-μl reaction contained 9 μCi of [^{35}S]methionine (approximately 0.3 μM final concentration) and 0.4 A_{260} unit of total bovine pituitary RNA or 0.06 A_{260} unit of poly(A)-containing bovine pituitary RNA. The poly(A)-containing RNA was prepared from the total RNA fraction by oligo(dT)-cellulose chromatography (15).

Cotranslational Cleavage of Nascent Presecretory Proteins. Two different procedures were used for the cotranslational assay of signal peptidase. In both procedures, cell-free translation and cleavage of presecretory proteins are concurrent events. In the first procedure, EDTA-stripped total or rough microsomes were added to the cell-free system, kept between 0° and 4°, prior to the start of protein synthesis, and *in vitro* translation was allowed to proceed at 25° for 90 min. In the second procedure, aliquots of the detergent extract were added to a modified cell-free translation mixture. The modification consisted of the addition of the nonionic detergent Nikkol (octa-ethylene glycol dodecyl ether) to a final concentration of 0.016 M. Addition of the second detergent was necessary to decrease deoxycholate inhibition of the cell-free translation (unpublished data).

Post-Translational Cleavage of Presecretory Proteins. The [^{35}S]methionine-labeled presecretory proteins used in the post-translational signal peptidase assay were synthesized *in vitro* in the wheat germ system, frozen, and stored at −20° for future use. A typical 50-μl post-translational cleavage assay contained 20 μl of cell-free translation mixture (containing the [^{35}S]methionine-labeled presecretory proteins), 10 μl of water, and 20 μl of the detergent extract. Cleavage was allowed to proceed at 25° for 90 min. (The time, temperature, pH, and detergent concentration of the post-translational cleavage assay have been optimized by independently varying each of these parameters.) The assay was terminated by the addition of 1 volume of ice-cold 10% (wt/vol) trichloroacetic acid.

Products of both the cotranslational and post-translational cleavage reactions as well as the uncleaved translation products themselves were analyzed by electrophoresis in linear 10–15% polyacrylamide gradient slab gels containing 0.1% $NaDodSO_4$. The gels were stained, destained, dried, and subjected to autoradiography (10). The amino-terminal sequence of radiolabeled prolactin obtained by post-translational cleavage of preprolactin was determined as described (2).

We used linear 15–20% polyacrylamide slab gels containing urea as well as $NaDodSO_4$ (16) to detect the putative signal peptides that would be expected to be generated by an endoproteolytic cleavage of the presecretory proteins. The ratio of acrylamide to bisacrylamide in the 15–20% resolving gel was 68.2:1.0. The resolving gel also contained 0.45 M Tris·HCl (pH 8.8), 0.1% $NaDodSO_4$, and 8 M urea, as well as a 0–10% linear gradient of sucrose. The 1.2-mm-thick resolving gels were 20 cm in length with a 2-cm stacking gel. The stacking gel contained 5% acrylamide, 0.13% bisacrylamide, 67 mM Tris·HCl (pH 6.8), and 0.1% $NaDodSO_4$. The electrode buffers consisted of 25 mM Tris base/12 mM glycine, pH 8.2/0.1% $NaDodSO_4$. In preparation for electrophoresis, the trichloroacetic acid-precipitated samples were resuspended in 32% sucrose/75 mM Tris base/0.001% bromphenol blue/4.5% $NaDodSO_4$/11 mM dithiothreitol, boiled for 2 min, cooled to room temperature, and alkylated by the addition of 1/5 volume of 0.25 M α-iodoacetamide.

Source of Materials. [^{3}H]Leucine (80 Ci/mmol), [^{3}H]proline (60 Ci/mmol), and [^{35}S]cystine (28 Ci/mmol) were obtained from New England Nuclear, Boston, MA. [^{35}S]methionine, (500–1000 Ci/mmol) was purchased from Amersham/Searle, Arlington Heights, IL. Nikkol is a product of Nikko Chemical Co., Tokyo, Japan. Sodium deoxycholate was obtained from Schwarz/Mann, Orangeburg, NY. Triton X-100 was from New England Nuclear. Wheat germ was obtained from Pillsbury Co., Minneapolis, MN. Apomyoglobin was obtained from Beckman Instruments, Inc., Palo Alto, CA. Bovine pituitary RNA and poly(A)-containing bovine pituitary RNA (both prepared from anterior lobes only) were a generous gift from V. Lingappa.

RESULTS

Cotranslational and Post-Translational Processing of Presecretory Proteins. The detergent extract prepared from dog pancreas total microsomes was assayed for its ability to reduce the size of preprolactin and pre-growth-hormone to that of prolactin and growth hormone, respectively (Fig. 1). The extract was assayed both *during in vitro* protein synthesis and *after* synthesis had been completed. As demonstrated in Fig. 1 (lane 1), preprolactin and pre-growth-hormone were the major products of the cell-free translation of bovine pituitary poly(A)-containing RNA. Lingappa *et al.* (1) have previously shown that, when dog pancreas stripped microsomes are added to the wheat germ system during translation, a portion of the nascent preprolactin and nascent pre-growth-hormone are cleaved to the authentic hormones. This result was confirmed (Fig. 1, lane 2). When added during translation, the detergent extract also was capable of producing polypeptides with the mobility of prolactin and growth hormone (Fig. 1, lane 3). When this same extract was added to an aliquot of the cell-free translation mixture after translation had been allowed to proceed to completion (i.e., to a sample containing full-length preprolactin and pre-growth-hormone molecules), it still produced molecules with the mobility of prolactin and growth hormone (Fig. 1, lane 5). In contrast, an aliquot of the cell-free translation mixture incubated in the absence of the detergent extract did not produce any molecules with the mobility of prolactin and growth hormone (Fig 1, lane 4). We confirmed that the post-translational cleavage shown in Fig. 1 was not an artefact due to residual translation by the now diluted *in vitro* translation system by conducting the post-translational cleavage assay in the presence of increasing amounts of cycloheximide. At a concentration of 2 μg/ml, cycloheximide completely inhibited cell-free translation; however, the post-translational

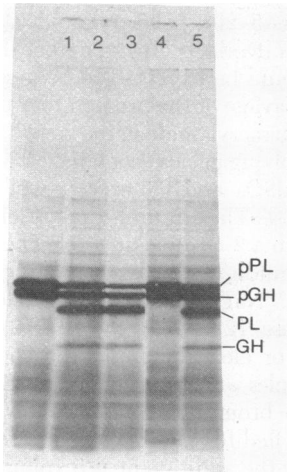

FIG. 1. Cotranslational and post-translational cleavage of preprolactin and pre-growth-hormone by signal peptidase. Poly(A)-containing mRNA from bovine pituitary was translated in the wheat germ cell-free system either in the absence (lane 1) or in the presence (lane 2) of EDTA-stripped total microsomes (1.4 A_{280} units/ml) or in the presence (lane 3) of a deoxycholate extract of total microsomes (0.95 A_{280} unit/ml), supplemented with the nonionic detergent Nikkol. Those translation products synthesized in the absence of either membranes or extract (see lane 1) were subsequently incubated for 90 min at 25° in the absence (lane 4) or in the presence (lane 5) of a deoxycholate extract of total microsomes (3.6 A_{280} units/ml). Analysis was by polyacrylamide gel electrophoresis in $NaDodSO_4$ and subsequent autoradiography. pPL, Preprolactin; PL, prolactin; and pGH, pre-growth-hormone; GH, growth hormone.

cleavage reaction was unaffected by cycloheximide at 10 μg/ml, the highest concentration tested (data not shown).

Fig. 2 demonstrates that the post-translational cleavage reaction is temperature sensitive. Samples of translation products and detergent extract, both at 0°, were mixed and subsequently

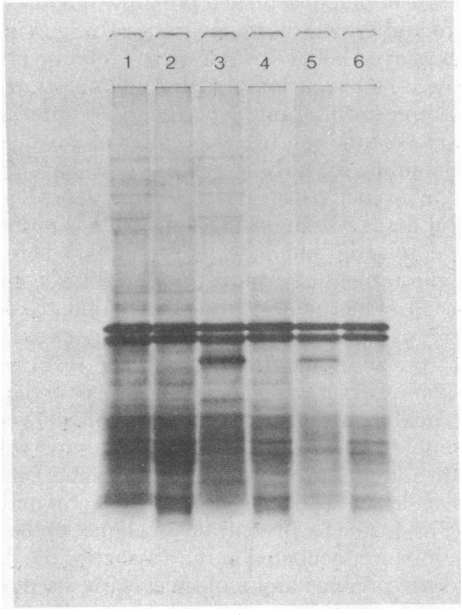

FIG. 2. Temperature sensitivity of solubilized signal peptidase. Preprolactin and pre-growth-hormone (see Fig. 1) were incubated post-translationally for 90 min at various temperatures either in the absence (lanes 2, 4, and 6) or in the presence (lanes 1, 3, and 5) of a deoxycholate extract of rough microsomes (3.6 A_{280} units/ml). Incubation was at 0° (lanes 1 and 2); at 25° (lanes 3 and 4); and at 37° (lanes 5 and 6).

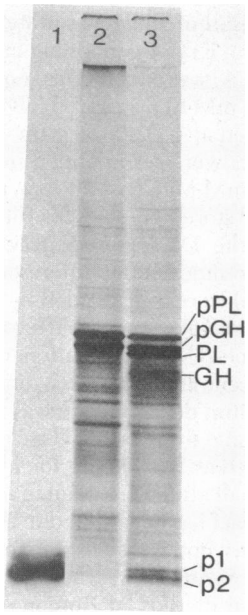

FIG. 3. Small peptides generated by cotranslational cleavage of nascent preprolactin and pre-growth-hormone. Poly(A)-containing mRNA from bovine pituitary was translated in the wheat germ cell-free system in the absence (lane 2) or in the presence (lane 3) of EDTA-stripped rough microsomes (2.55 A_{280} units/ml). Translation products were analyzed by gel electrophoresis in urea and $NaDodSO_4$, followed by autoradiography. Lane 1 contained ^{125}I-labeled bovine glucagon as a molecular weight marker. Designations are as in Fig. 1. p1 and p2, Small peptides.

incubated at 0°, 25°, or 37° for 90 min. At 0° the presecretory proteins were not cleaved (Fig. 2, lane 2). At 37° there was some cleavage (Fig. 2, lane 5), but it was considerably less than the amount of cleavage obtained at 25° (Fig. 2, lane 3). Subsequent experiments (data not shown) in which the detergent extract was incubated at 0°, 25° or 37° for increasing amounts of time and assayed post-translationally for signal peptidase at 25° revealed that the signal peptidase is rapidly inactivated at 37°. The small amount of activity that remained after 10 min at 37° was completely lost by 20 min of incubation. On the other hand, incubation at 0° or 25° for up to 90 min did not affect the signal peptidase activity of the extract. The cause of this rapid inactivation of signal peptidase in the extract is not clear.

A question that remained to be resolved was whether the signal peptidase is an endo- or an exoproteolytic enzyme. If the signal peptidase is an endoprotease, cotranslational or post-translational cleavage of presecretory proteins should generate both the authentic secretory protein and its signal peptide. In an attempt to detect the signal peptides generated by cotranslational cleavage of preprolactin and pre-growth-hormone with EDTA-stripped microsomes, we examined the products by polyacrylamide gel electrophoresis in urea as well as $NaDodSO_4$. The results of this experiment are presented in Fig. 3. In the membrane-containing sample (Fig. 3, lane 3) we detected two small peptides (designated p1 and p2) with electrophoretic mobilities comparable to those of bovine glucagon (Fig. 3, lane 1) that were not present in the control sample (Fig. 3, lane 2). On the basis of their electrophoretic mobility and their appearance only in samples whose presecretory proteins have been cleaved, we believe that these two peptides could be the signal peptide portion of preprolactin and pre-growth-hormone. However, definitive identification must await sequence analysis of the peptides.

Subcellular Localization of Signal Peptidase. The exper-

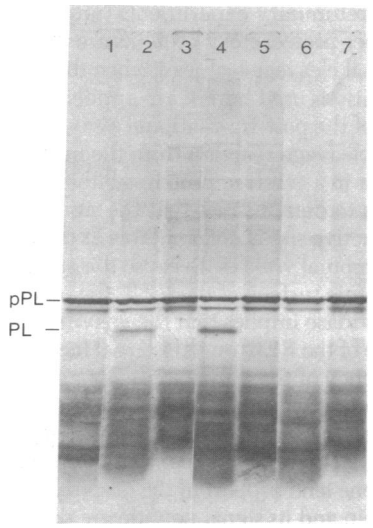

FIG. 4. Signal peptidase is located in the rough microsomes. Preprolactin and pre-growth-hormone, synthesized by translation of total pituitary RNA in the wheat germ cell-free system, were incubated post-translationally for 90 min at 25° in the absence (lane 1) or in the presence of various membrane fractions or their deoxycholate extracts: lane 2, extract of total microsomes (3.4 A_{280} units/ml); lane 3, EDTA-stripped total microsomes (25.2 A_{280} units/ml); lane 4, extract of rough microsomes (2.4 A_{280}/ml); lane 5, EDTA-stripped rough microsomes (20.4 A_{280} units/ml); lane 6, extract of smooth microsomes (2.3 A_{280} units/ml); lane 7, smooth microsomes (8.4 A_{280} units/ml). The pre-growth-hormone band is less prominent when the cell-free translation system is directed by unfractionated RNA than when it is directed by poly(A)-containing RNA (compare lanes 1 of Figs. 1 and 3).

iment described in Fig. 1 was conducted with a detergent extract prepared from total dog pancreas microsomes, a fraction containing smooth as well as rough microsomes. If the activity that we observed indeed were due to signal peptidase, it would be expected to be localized in the rough microsomal fraction which is comprised primarily of derivatives of the RER. To investigate the subcellular localization of the activity, deoxycholate extracts of purified rough and smooth microsomes as well as total microsomes were assayed for their ability to cleave preprolactin post-translationally. Fig. 4 presents the results of this experiment. As expected the activity was present in the deoxycholate extracts of total microsomes (lane 2) and rough microsomes (lane 4) but not in the smooth microsomal extract (lane 6).

In addition, Fig. 4 provides information regarding the localization of the signal peptidase with respect to the rough microsomal membranes. EDTA-stripped total microsomes and rough microsomes, both of which are capable of cleaving nascent presecretory polypeptides cotranslationally (refs. 1, 2, 11, 12, and 14; Fig. 1), were inactive in the post-translational assay (Fig. 4, lanes 3 and 5, respectively). However, deoxycholate extracts of these membranes were capable of cleaving preprolactin to prolactin in the post-translational assay (Fig. 4, lanes 2 and 4). This demonstrates the latency of the signal peptidase and clearly indicates that the active site of the enzyme is unavailable at the exterior surface of the stripped microsomal vesicles, which corresponds to the cytoplasmic surface of the RER. This implies that the active site of signal peptidase is located either on the cisternal face of the RER membrane, within the membrane itself, or, perhaps, in the cisterna of the RER.

Fidelity of Cleavage by Detergent-Solubilized Signal Peptidase. Definitive evidence that the extracted protease is signal peptidase and that it retains its substrate specificity was

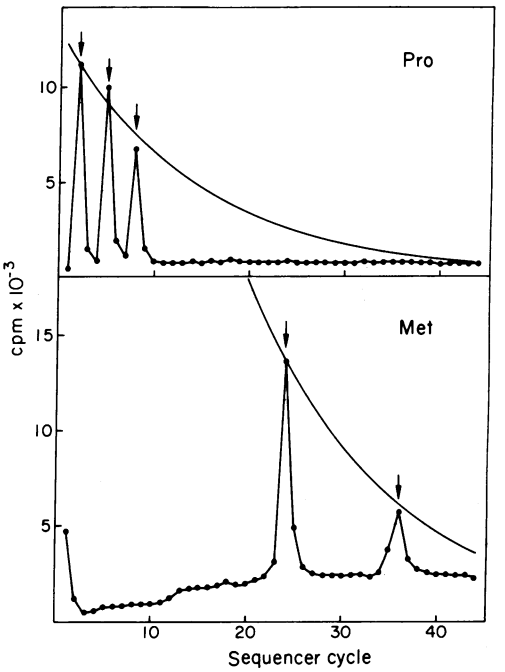

FIG. 5. Partial amino-terminal sequence analysis of post-translationally cleaved preprolactin. Total bovine pituitary RNA was translated in a wheat germ cell-free system containing 462 μCi of [^{35}S]methionine (740 Ci/mmol) and 150 μCi of [^{3}H]proline (60 Ci/mmol) in a final volume of 2.0 ml. After completion of translation, a deoxycholate extract prepared from rough microsomes was added and incubation was continued for another 90 min at 25°. After gel electrophoresis and autoradiography, the processed prolactin was subjected to 44 cycles of automated Edman degradation. The recovered thiazolinones were dried, and their ^{3}H (*Upper*) and ^{35}S (*Lower*) radioactivities were determined. The ^{3}H radioactivity was corrected for a 13% spill from the ^{35}S channel. The smooth curved line represents a theoretical repetitive yield of 93.5%, normalized to the first radioactive peak.

obtained by partial amino-terminal sequence analysis of the prolactin produced by post-translational cleavage of preprolactin. Preprolactin was labeled with [^{35}S]methionine and [^{3}H]proline or with [^{35}S]cysteine and [^{3}H]leucine and then post-translationally cleaved with a deoxycholate extract of rough microsomes. The products were analyzed by gel electrophoresis in $NaDodSO_4$ followed by autoradiography. The band with the mobility corresponding to that of prolactin was excised and sequenced. As shown in Figs. 5 and 6, the positions of proline (cycles 2, 5, and 8), methionine (cycles 24 and 36), cysteine (cycles 4 and 11), and leucine (cycles 15 and 18) in the putative prolactin are identical to the positions of these amino acids in the known sequence of bovine prolactin (17). From this we conclude that the post-translational cleavage of preprolactin by the deoxycholate extract of dog pancreas rough microsomes produces a prolactin molecule with the correct amino-terminal sequence.

DISCUSSION

Our results demonstrate that the signal peptidase can be solubilized from dog pancreas rough microsomal membranes with the detergent deoxycholate. The solubilized signal peptidase retains its ability to cleave bovine preprolactin and pre-growth-hormone cotranslationally to prolactin and growth hormone, respectively. More importantly, we have shown that the solubilized signal peptidase is capable of processing full-length preprolactin and pre-growth-hormone molecules post-translationally. In the case of preprolactin, we have shown, by

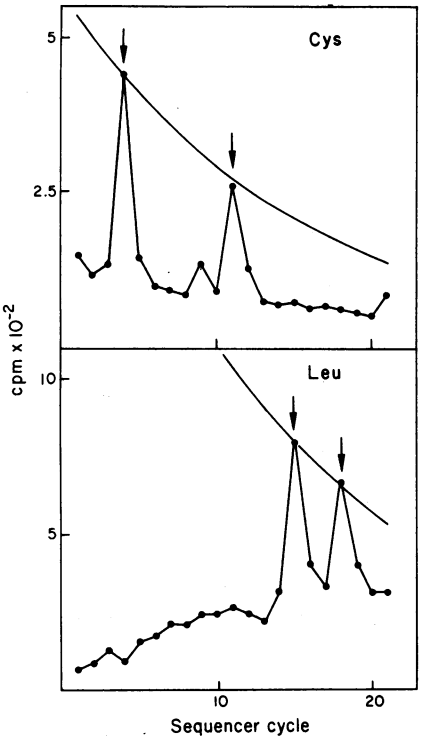

FIG. 6. Partial amino-terminal sequence of post-translationally cleaved preprolactin. Total bovine pituitary RNA was translated in a wheat germ cell-free system (1.0 ml final volume) containing 115 μCi of [^{35}S]cysteine (13.8 Ci/mmol) and 50 μCi of [^{3}H]leucine (80 Ci/mmol). The products were post-translationally cleaved with a deoxycholate extract prepared from rough microsomes (3.6 A_{280} units/ml, final concentration) and the prolactin band was sequenced as described in the legend to Fig. 5. The ^{35}S (*Upper*) and ^{3}H (*Lower*) radioactivities of the recovered thiazolinones were determined. The ^{3}H radioactivity was corrected for a 13% spill from the ^{35}S channel. The smooth curved line represents a theoretical repetitive yield of 93.5%, normalized to the first radioactive peak.

partial amino-terminal sequence analysis, that the prolactin produced by post-translational cleavage has the amino-terminal sequence of authentic prolactin. Although it has been shown (1, 2, 12) that signal peptidase contained in undissociated microsmal vesicles can cotranslationally cleave nascent presecretory proteins at the correct site, our demonstration here that the solubilized signal peptidase retains its site-specific activity was necessary, because otherwise it could be argued that the solubilized enzyme is not signal peptidase but some other protease associated with dog pancreas rough microsmal vesicles or, less likely, that detergent-solubilized signal peptidase assumes an altered site specificity. However, it must be emphasized that the ability of the solubilized signal peptidase to cleave full-length preprolactin and pre-growth-hormone correctly in the post-translational assay should not be construed to imply that this reaction also occurs post-translationally *in vivo*. Experiments in which incomplete polypeptide chains from detached polysomes were completed *in vitro* have clearly established that *in vivo* cleavage occurs cotranslationally on nascent (i.e., incomplete) presecretory proteins. In fact, our inability to cleave every preprolactin molecule in the post-translational assay (we cleave maximally 50% of the molecules) may indicate that a fraction of the preprolactin molecules have folded in such a manner that their cleavage site is not accessible. In support of this view, preliminary experiments (unpublished data) indicate that other presecretory proteins are poor substrates for post-translational cleavage—at least when the post-translational cleavage conditions used herein are applied without change.

The utility of the post-translational assay lies in its ability to dissociate the cleavage reaction from the multiplicity of reactions that occur in a cell-free protein-synthesizing system. This has allowed us to demonstrate that the signal peptidase is latent—i.e., its active site is not available at the exterior surface of rough microsomal vesicles. Because this surface corresponds to the cytoplasmic surface of the RER, the observed latency of the signal peptidase implies that the enzyme is located within the membrane of the RER, on its cisternal face, or in the cisterna itself. The latency of the signal peptidase and our detection of two small peptides with the expected characteristics (size and appearance only in cleaved samples) of the endoproteolytically generated signal peptide portions of preprolactin and pre-growth-hormone lend support to the proposal (10) that both the secreted protein and its signal peptide are discharged together into the cisternae of the RER.

Perhaps most important, the ability to assay signal peptidase post-translationally, unencumbered by the complexity and restrictions imposed by *in vitro* protein synthesis, will facilitate our ongoing attempts to purify and characterize this enzyme.

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