PARTIAL AMINO-TERMINAL SEQUENCE OF CELL-FREE TRANSLATION PRODUCT ENCODED BY BOVINE CORTICOTROPIN-β-LIPOTROPIN PRECURSOR MESSENGER RNA

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1. Introduction

Recently, we have determined the complete nucleotide sequence of a cloned cDNA insert that contains a duplex copy of the mRNA coding for the bovine corticotropin–β-lipotropin precursor [1]. The nucleotide sequence determined has defined the precise locations of corticotropin and β-lipotropin in the precursor protein and has predicted the amino acid sequence of its remaining portion. On the basis of our finding that the precursor protein is composed of several repetitive units plus a putative signal peptide with a high content of hydrophobic amino acids, we have proposed that the translational initiation site is located at the methionine residue at position −131 [1]; the amino acid residues on the amino-terminal side of corticotropin, which have been predicted from the nucleotide sequence of the cDNA, are indicated by negative numbers. The present investigation was designed to verify this proposal by determining the partial amino acid sequence of the amino-terminal region of the cell-free translation product encoded by the corticotropin–β-lipotropin precursor mRNA. The results obtained have demonstrated that the partial amino-terminal sequence determined agrees with the amino acid sequence predicted from the nucleotide sequence of the cDNA, thus verifying the proposed location of the translational initiation site.

2. Materials and methods

2.1. *Cell-free translation and immunoprecipitation*

RNA was isolated from the membrane fraction of neurointermediate lobes of bovine pituitaries, and poly(A)-containing RNA was prepared by oligo(dT)-cellulose chromatography as in [2]. The poly(A)-containing RNA was translated in a cell-free protein-synthesizing system derived from rabbit reticulocytes as in [3] with the following modifications. The reticulocyte lysate was incubated with micrococcal nuclease (Worthington, Freehold) to destroy endogenous mRNA activity [4] and was chromatographed on Sephadex G-25 (Pharmacia, Uppsala) to reduce the free amino acid content [5]. The reaction mixture (total vol. 0.5 ml) containing 0.3 ml nuclease- and Sephadex G-25-treated reticulocyte lysate, 0.25–0.5 mCi L-[4,5-^{3}H]leucine (137 Ci/mmol) or L-[2,3,4,5-^{3}H]proline (117 Ci/mmol) (Radiochemical Centre, Amersham), 10 μg poly(A)-containing RNA and the remaining components at the concentrations described [3] was incubated at 25°C for 90 min in the presence of 90 μg pig heart citrate synthase (Boehringer, Mannheim) and 0.5 μmol potassium oxaloacetate, which were added to prevent possible acetylation [6]. The translation product was subjected to indirect immunoprecipitation with antibody specific to corticotropin as in [2]. The immunoprecipitated translation product was analyzed by electrophoresis on discontinuous sodium dodecyl-sulfate–polyacrylamide slab gel followed by fluorography according to [7].

2.2. *Radiosequencing by automated Edman degradation*

The immunoprecipitated translation product, mixed with 5 mg sperm whale apomyoglobin (Wako Pure Chemical, Osaka), was heated at 95°C for 10 min

in 0.5 ml 0.5% sodium dodecylsulfate and then applied to a JEOL Sequence Analyzer JAS-47K (Tokyo). Automated sequencing was performed according to [8] with the use of the standard JEOL Quadrol program modified for double phenylisothiocyanate coupling and double heptafluorobutyric acid cleavage during the first sequenator cycle. One-half of the thiazolinone in the 1-chlorobutane extract from each step was dried under nitrogen, and the radioactivity was determined with a liquid scintillation spectrometer as in [2]. The remainder of each thiazolinone was dried under nitrogen and converted to its phenylthiohydantoin by heating in 1 M HCl at 80°C for 10 min. The repetitive yield of amino acids released from apomyoglobin carrier was determined by gas–liquid chromatography as in [8] to monitor the sequenator for proper operation.

3. Results and discussion

Poly(A)-containing RNA from neurointermediate lobes of bovine pituitaries was translated in a cell-free protein-synthesizing system in the presence of radioactive amino acids. The translation product encoded by the corticotropin–β-lipotropin precursor mRNA was isolated by indirect immunoprecipitation with antibody specific to corticotropin. Analysis of the immunoprecipitated translation product by sodium dodecylsulfate–polyacrylamide gel electrophoresis revealed a single radiolabelled component as described [3]. The partial amino-terminal sequence of the translation product of the corticotropin–β-lipotropin precursor mRNA was determined by subjecting immunoprecipitated samples to radiosequencing by automated Edman degradation. Figure 1A shows the results of 25 consecutive Edman degradations of the translation product labelled with [^{3}H]leucine. Radioactivity peaks appeared at cycles 3, 11, 12, 13, 15, 16 and 17. As expected from sequenator analysis, the recovery of radioactivity decreased with increasing cycles, and there was always some trailing of radioactivity from the previous cycle because of incomplete degradation. Accordingly, the radioactivities at cycles 13, 16 and 17 were slightly higher than those at cycles 12, 15 and 16, respectively. The results obtained with the [^{3}H]proline-labelled translation product are presented in fig.1B. A radioactivity peak

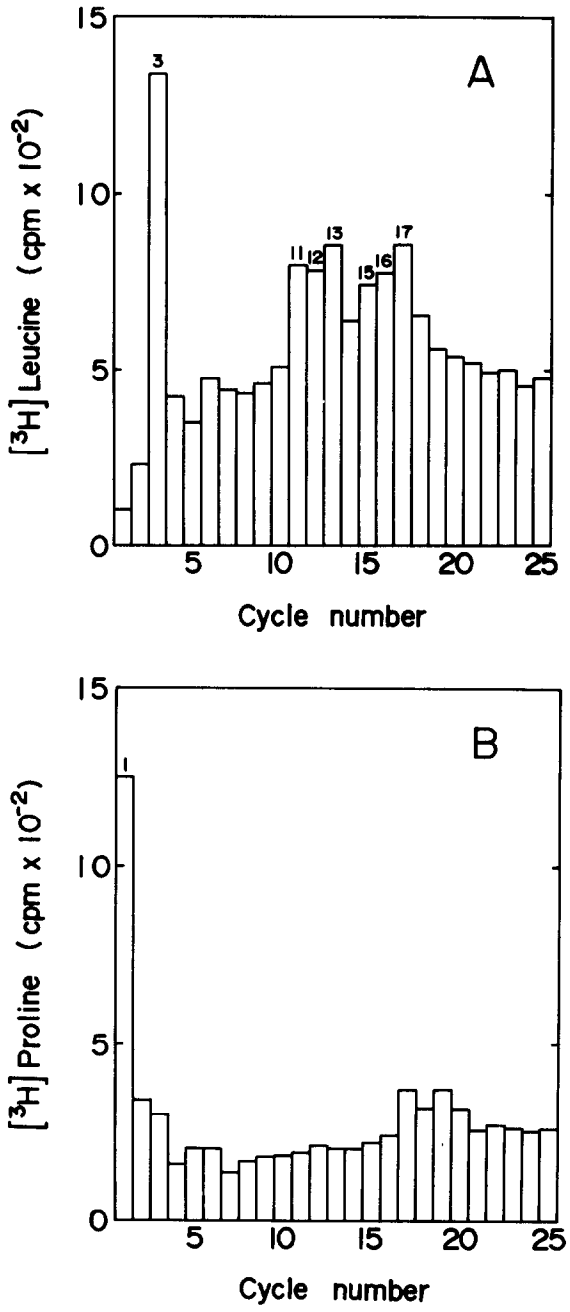

Fig.1. Sequential Edman degradation of the immunoprecipitated cell-free translation product labelled with [^{3}H]leucine (A) or [^{3}H]proline (B). The radioactivity applied to the sequenator was 1.2 × 10^5 cpm (A) or 1.5 × 10^5 cpm (B). The repetitive yield of amino acids released from apomyoglobin carrier was 89% for both A and B. The numbers over the histogram indicate sequenator cycle.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A		Pro	X	Leu	X	X	X	X	X	X	Leu	Leu	Leu	X	Leu	Leu	Leu
B	Met	Pro	Arg	Leu	Cys	Ser	Ser	Arg	Ser	Gly	Ala	Leu	Leu	Leu	Ala	Leu	Leu	Leu

Fig.2. Comparison of the radiosequencing data (A) with the amino acid sequence of the amino-terminal region of the bovine corticotropin–β-lipotropin precursor predicted from the nucleotide sequence of its cDNA (B). The numbers indicate sequenator cycle. The sequence of B has been taken from [1].

appeared at cycle 1 of Edman degradation. From these data, we conclude that the amino-terminal region of the cell-free translation product of the bovine corticotropin–β-lipotropin precursor mRNA has the following partial sequence:

$$\overset{1}{\text{Pro}}\text{–X–}\overset{3}{\text{Leu}}\text{–X–X–X–X–X–X–X–}\overset{11}{\text{Leu}}\text{–}\overset{12}{\text{Leu}}\text{–}$$

$$\overset{13}{\text{Leu}}\text{–X–}\overset{15}{\text{Leu}}\text{–}\overset{16}{\text{Leu}}\text{–}\overset{17}{\text{Leu}}$$

As shown in fig.2, the partial amino acid sequence determined agrees with the sequence of the amino-terminal region of the bovine corticotropin–β-lipotropin precursor predicted from the nucleotide sequence of its cDNA [1], provided that the initiative methionine residue is removed from the predicted sequence. The removal of the initiative methionine residue during cell-free protein synthesis has been reported for other eukaryotic proteins [9–11]; actually, we confirmed that the initiative methionine residue of rabbit globin was eliminated in the cell-free translation system used in the present study. Thus, the results presented verify the location of the translational initiation site proposed [1].

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