Partial Amino Acid Sequence of Rat Pre-prolactin

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Rat pituitary mRNA was used to direct the cell-free synthesis of pre-prolactin labelled with [4,5-³H]leucine and either [³⁵S]methionine or [³⁵S]cystine. Sequence analysis of the labelled protein indicates that pre-prolactin has 29 amino acid residues joined to the *N*-terminus of the prolactin sequence. Leucine residues were found at positions 13, 14, 15, 16, 21 and 22, methionine residues at positions 1, 17 and 18, and a cysteine residue at position 24 of the precursor sequence, and this partial sequence shows considerable similarity with other precursors that have been sequenced.

Translation of mRNA in cell-free systems has demonstrated that a number of secretory proteins are synthesized as species of higher molecular weight than the normally secreted form of the protein (Swan *et al.*, 1972; Kemper *et al.*, 1974; Boime *et al.*, 1975; Suchanek *et al.*, 1975; Devillers-Thiery *et al.*, 1975; Maurer *et al.*, 1976; Sussman *et al.*, 1976; Beato & Nieto, 1976). The use of proteinase inhibitors has also allowed the detection of these high-molecular-weight species in intact cells (Sussman *et al.*, 1976; Schmeckpeper *et al.*, 1975), suggesting that they are short-lived precursors, which are enzymically cleaved before secretion. The precursor segment of these secretory proteins may have unique properties that fulfil some function in the synthesis and secretion of the protein. Therefore we decided to determine the amino acid sequence of these proteins. We report here the partial amino acid sequence of rat pre-prolactin, a high-molecular-weight form of prolactin.

Materials and Methods

[4,5-³H]Leucine (50 Ci/mmol), [³⁵S]methionine (400 Ci/mmol) and [³⁵S]cysteine (55 Ci/mmol) were from New England Nuclear, Boston, MA, U.S.A.

Rat pituitary mRNA was obtained by homogenization of pituitaries in a buffer containing sodium lauryl sarcosinate, sedimentation through CsCl cushions and chromatography on oligo(dT)-cellulose as described by Maurer *et al.* (1976). This RNA was layered over 11.2 ml 5–20% (w/v) sucrose gradients containing 0.01 M-Tris/HCl, pH 7.4, 1 mM-EDTA and sodium dodecyl sulphate (5 g/l) and centrifuged at

201 000g (*r*_{av}, 10.78 cm) for 6 h at 15°C in an SW 41 rotor (Beckman Instruments, Palo Alto, CA, U.S.A.). Prolactin mRNA activity sedimenting between 4 and 18S was pooled.

This mRNA was used to direct protein synthesis in the cell-free wheat-germ system and pre-prolactin was isolated by immunoprecipitation as described previously (Maurer *et al.*, 1976). Cell-free translation products were labelled with [³H]leucine and [³⁵S]methionine or in separate experiments with [³H]leucine and [³⁵S]cystine. Immunoprecipitates were dissolved in 0.4 ml of 6M-guanidine hydrochloride/0.05 M-Tris/HCl, pH 7.4, and chromatographed on a column (0.8 cm × 30 cm) of Sephadex G-25 in 0.05 M-NH₄HCO₃, pH 8. The radioactive material eluted in the void volume was used for sequencing.

Labelled pre-prolactin was subjected to repetitive Edman degradation by using an automated sequenator, and the data were corrected for background and repetitive yield. The general methods used for the sequence determination have been described in detail previously (Smithies *et al.*, 1971; McKean *et al.*, 1974).

Results and Discussion

Pre-prolactin labelled with [³H]leucine and either [³⁵S]methionine or [³⁵S]cystine was sequenced for 50 cycles in an automated sequenator. Major peaks of radioactivity from methionine residues were found after cycles 1, 17 and 18, from cysteine after cycles 24, 33 and 38, and from leucine after cycles 13, 14, 15, 16, 21, 22, 30, 42 and 45 (Fig. 1). The sequence of these amino acids in pre-prolactin that these results suggest is summarized at the bottom of Fig. 1, along with the sequence of these same amino acids at the *N*-terminus

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Fig. 1. *Sequence analysis of pre-prolactin labelled with [^3H]leucine and either [^{35}S]methionine or [^{35}S]cystine* Labeled pre-prolactin was sequenced for 50 cycles in an automated sequenator and the data were corrected for repetitive yield and background. To help eliminate background radioactivity, cycle zero did not include phenyl isothiocyanate. Also, the derived structure of pre-prolactin and the published sequence of these same amino acids at the *N*-terminus of prolactin (Parlow & Shome, 1976) are summarized. Abbreviations: L, leucine; M, methionine; C, cysteine; X, some unidentified amino acid. (a) Methionine; (b) cysteine; (c) leucine.

of prolactin (Parlow & Shome, 1976). The leucine residues at positions 30, 42 and 45 and cysteine residues at positions 33 and 38 align with the sequence of prolactin. This suggests that pre-prolactin contains

a 29-amino acid addition preceding the prolactin sequence.

Analysis of the smaller peaks of radioactivity (Fig. 1) suggests the presence of minor sequences which

the prolactin sequence in order to protect the peptide bond connecting the precursor peptide to the prolactin sequence until a particular stage in synthesis when a disulphide rearrangement might occur (DeLorenzo *et al.*, 1966). Cysteine residues have not been investigated in the sequence of any other precursor proteins.

Comparison of the sequence of the precursor segment of pre-prolactin with the published sequence of other precursors reveals similarities in the leucine-sequence pattern (Fig. 2). This similarity in the very unusual leucine-sequence pattern of these proteins occurs despite the fact that the ultimate biological activities of the proteins are quite different and the fact that the proteins are from a number of different animal species. It is clear that final sequence comparison of these precursors will require determination of their complete amino acid sequences. However, the similarities in the hydrophobic nature of the amino acid sequences are consistent with a common role for the precursors. The hydrophobic precursor segment should favour interactions with membranes. Thus polyribosomes synthesizing secretory proteins may bind to membranes of the endoplasmic reticulum because of a hydrophobic precursor segment of the nascent protein chain. Subsequently, the protein is transported into the cisternae of the endoplasmic reticulum, where the precursor segment may be removed. The demonstration that membrane preparations can remove the precursor segment is consistent with this model (Blobel & Dobberstein, 1975; Szczesna & Boime, 1976).

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