Vol. 87, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 27, 1979 Pages 1006-1010

A NEW GLYCOPEPTIDE IN PIG, OX AND SHEEP PITUITARY

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Received March 5,1979

SUMMARY

A novel 39 residue glycopeptide has been isolated in substantial yield from pig, ox and sheep pituitary glands. The amino acid sequence is strongly conserved, suggesting that the peptide may fulfil an important role. The occurrence of two fragments of the pituitary glycopeptide which terminate in paired leucine residues reflects the action of a processing enzyme with previously unknown specificity.

INTRODUCTION

During the course of a study on prohormone activation, four novel peptides were isolated from porcine pituitary glands (1). Three were seen to have their origin in known pituitary polypeptides but the fourth, a glycopeptide, could not be aligned with a specific precursor. The glycopeptide was accompanied by significant amounts of two constituent peptides, which were of particular interest as their C-terminal sequences appeared to reflect the action of processing enzymes with unusual specificity. We report here the primary structure of the 39 residue glycopeptide which has now been obtained from the pituitary of three species. It exhibits remarkable sequence conservation.

METHODS

The porcine, bovine and ovine glycopeptides were extracted from pituitary glands in acid acetone by a

0006-291X/79/081006-05$01.00/0

procedure described previously (2). The mixture of peptides not retained on CM-cellulose at pH 5.0 or on DEAE-Sephadex A25 at pH 8.0 was resolved by gradient elution from SP-Sephadex C25 at pH 4.0 and by gel filtration on Sephadex G50, providing the homogeneous glycopeptide. For sequence analysis, the glycopeptides were cleaved with trypsin to give three fragments, residues 1-4, 5-20 and 21-39, which were separated by gel filtration on Sephadex G50. The larger peptides were digested with papain and the products resolved on Sephadex G25 and on columns of IR-120. Stepwise degradation was performed by the Edman procedure and by digestion with aminopeptidase M and carboxypeptidase A, which also allowed assignment of the amides. Confirmation of the sequences was obtained by applying the same techniques to fragments of the glycopeptides that were isolated from the glands. The oligosaccharide attached to the asparagine residue at position 6 was found to contain 5 residues of glucosamine; other carbohydrates were not determined.

RESULTS AND DISCUSSION

Inspection of the linear sequence of amino acids in the glycopeptide (Fig.1) reveals that it contains two distinct regions. From positions 9 to 22 there is a preponderance of hydrophobic amino acids, with a high proportion of leucine residues; the remaining sequence is rich in proline, glycine, alanine and glutamic acid and probably exists as an extended chain. This arrangement of a hydrophobic region leading into an extended region of structure seems to be a feature of many hormone precursors (3-6).

The existence of two naturally occurring fragments of the glycopeptide, comprising residues 1 to 10 and 1 to 19, indicates that pituitary contains processing enzymes which act at the carboxyl side of paired leucine residues; and a similar fragment which corresponds to residues 1 to 18 of the glycopeptide has been reported in pituitary powders (7), again reflecting the same enzymic specificity. Two more peptides, which account for residues 23 to 39 and 26 to 39 of the sequence, were also isolated; their formation would seem to involve cleavage on the carboxyl side of

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         1                                   10                                  19
PIG      Ala Ser Asp Arg Ser Asn Ala Thr Leu Leu Asp Gly Pro Ser Gly Ala Leu Leu Leu Arg-
                             |
                             C
OX       Ala Asn Asp Arg Ser Asn Ala Thr Leu Leu Asp Gly Pro Ser Gly Ala Leu Leu Leu Arg-
                             |
                             C
SHEEP    Ala Ser Asp Arg Ser Asn Ala Thr Leu Leu Asp Gly Pro Ser Gly Ala Leu Leu Leu Arg-
                             |
                             C

                  23         26                                                    39
PIG      -Leu Val Gln Leu Ala Gly Ala Pro Glu Pro Ala Glu Pro Ala Gln Pro Gly Val Tyr

OX       -Leu Val Gln Leu Ala Gly Ala Pro Glu Pro Ala Glu Pro Ala Gln Pro Gly Val Tyr

SHEEP    -Leu Val Gln Leu Ala Ala Ala Pro Glu Pro Ala Glu Pro Ala Gln Pro Gly Val Tyr
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Fig. 1 Amino acid sequences of a glycopeptide isolated from pig, ox and sheep pituitary. Residues that differ are underlined. It was observed that the glutamine residue at position 23 undergoes deamidation to glutamic acid under mild acid conditions.

leucylvaline and alanine respectively. The former could be released by the enzyme that catalyses cleavage at consecutive hydrophobic residues while the latter appears to be formed by an enzyme with a specificity that is commonly involved in the early stages of posttranslational processing.

Several possibilities may be considered concerning the origin and role of the glycopeptide. It could form one section of a precursor to a pituitary hormone, either as the connecting peptide to a disulphide linked two chain hormone such as luteinizing hormone, follicle

stimulating hormone or thyroid stimulating hormone, or more simply as the terminal region of a single chain prohormone. In this context it is of interest that six consecutive residues of the porcine glycopeptide (positions 23 to 28) are identical to the six residues at the N-terminus of porcine lipotropin (8). Since lipotropin is formed from a biosynthetic precursor that has evolved by gene duplication (9,10), a plausible hypothesis is that the glycopeptide might originate from the carbohydrate containing N-terminal region of the corticotropin - lipotropin prohormone. However the porcine glycopeptide did not cross react with antibody raised against the N-terminal region of the rat prohormone. Another possible role for the glycopeptide is that it might represent the 'recognition site' of a larger, biologically active peptide in which a functional region is covalently attached to the address component. In any case, further studies are clearly needed to establish the biosynthetic origin of the glycopeptide and to determine whether it has a biological activity *per se*.

It is notable that the glycopeptide occurs in substantial quantity in the pituitary of three species. The strong conservation of its sequence would seem to imply that it fulfils an important biological role.

ACKNOWLEDGEMENT We thank Dr. Betty Eipper and Dr. Richard Mains of the University of Colorado for testing the immunoreactivity of the porcine glycopeptide with antisera raised against the N-terminal region of the rat ACTH-lipotropin prohormone.

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