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THE COMPLETE SEQUENCE OF A NOVEL HUMAN PITUITARY GLYCOPEPTIDE HOMOLOGOUS TO PIG POSTERIOR PITUITARY GLYCOPEPTIDE

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Summary

The isolation and complete purification of a novel human pituitary glycopeptide (HPGP) from whole pituitaries is described. Amino acid composition predicts a glycopeptide rich in glucosamine. Complete sequence determination showed it to be a 39 residues with an oligosaccharide chain attached at Asn residue No. 6. It exhibits marked sequence homology to previously isolated pig posterior pituitary glycopeptide and to whole pituitary glycopeptides isolated from ox, sheep and pig. Based on these results and the presence of such a peptide in posterior pituitary it is suggested that it could form part of the N-terminal glycopeptide extension of the precursor of neurophysin-arginine vasopressin.

Introduction

During the course of isolation and characterization of the N-terminal glycofragment of pro-opiomelanocortin (1,2) from human pituitaries, a novel glycopeptide was isolated. Its purification by high performance liquid chromatography led to its complete sequence determination. When aligned with peptides of known sequence it was found to be homologous to a glycopeptide isolated previously from posterior pituitary lobes of pig pituitaries (3). Recently a similar peptide, isolated from whole pituitary extracts of pig, ox and sheep, allowed the elucidation of the sequence of the peptides from these species (4). A high degree of homology between species was found in the sequence of this 39 residues glycopeptide (4). We report here the complete primary structure of the human analog. It also exhibits a striking sequence conservation and length, which could indicate a peptide of biological importance.

Methods

(a) Purification of glycopeptide from whole human pituitaries

Carboxymethyl cellulose chromatography of an HCl/Acetone extract of human pituitaries, allows the isolation of human β-lipotropin and β-endorphin, as late eluting peaks (5). The unretained peak on CM-cellulose was previously used as starting material for the high performance liquid chromatographic (HPLC) purifi-

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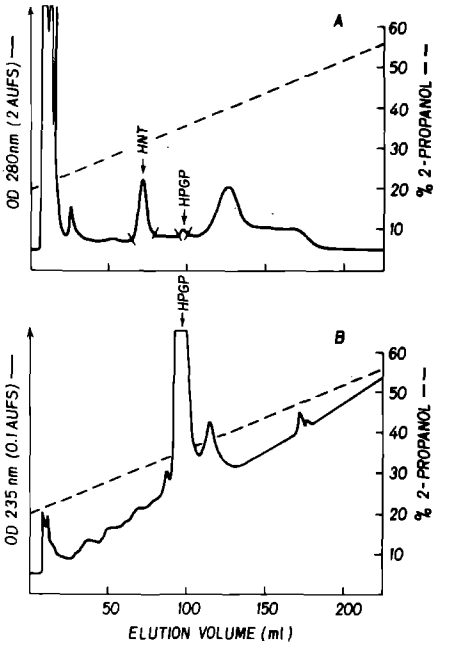

Fig. 1. High performance liquid chromatographic purification of the human pituitary glycopeptide (HPGP) from a human pituitary extract. The gradient of 2-propanol is shown as a dashed line. (A) Unretained peak on carboxymethylcellulose. (B) Repurification of material from peak HPGP in Fig. 1A, under the same conditions. Peak HNT in Fig. 1A represents the human N-terminal glycopeptide of pro-opiomelanocortin (1,2).

cation of the human N-terminal glycopeptide of pro-opiomelanocortin (1,2). The same procedure was used for the HPLC purification of the human glycopeptide reported here. The instrument consists of a Waters Model 240 liquid chromatograph equipped with a Model 730 data Module, a Model 720 system controller and a model 450 uv/vis variable detector. The column used was a Waters semipreparative μ-C18 column (0.7 x 30 cm). The eluant consisted of 0.02 M triethylamine formate pH 3.0 and 2-propanol. A linear gradient of 20% to 56% of 2-propanol with a duration of 80 min at 3 ml/min was used (Fig. 1A). The peak designated HPGP (human pituitary glycopeptide) in Fig. 1A was collected, lyophylized and then repurified under the same conditions (Fig. 1B). This provided a homogeneous preparation of the human glycopeptide.

(b) Amino acid analysis

Amino acid analysis was performed in triplicate following 24, 48 and 72 hrs 5.7 N HCl hydrolysis in vacuo at 105°C. The amino acid analyzer consisted of a modified Beckman 120C equipped with a model 126 computing integrator. The separation of the amino acids was done on a W3 resin (Beckman) which allows the separation of all the amino acids including the hexosamines (6). No correction was made for hexosamines destruction under these hydrolysis conditions.

(c) Sequence determination

The sequenator consisted of an updated Beckman 890B instrument equipped with a cold trap. The program used was a "0.3 M Quadrol pH 9.0 with S_1+S_2 wash" (Beckman #121178). For sequencing, 3.0 mg of Polybrene (Aldrich) + 100 nMoles of a dipeptide Ile-Val were first added to the cup and four dummy cycles were

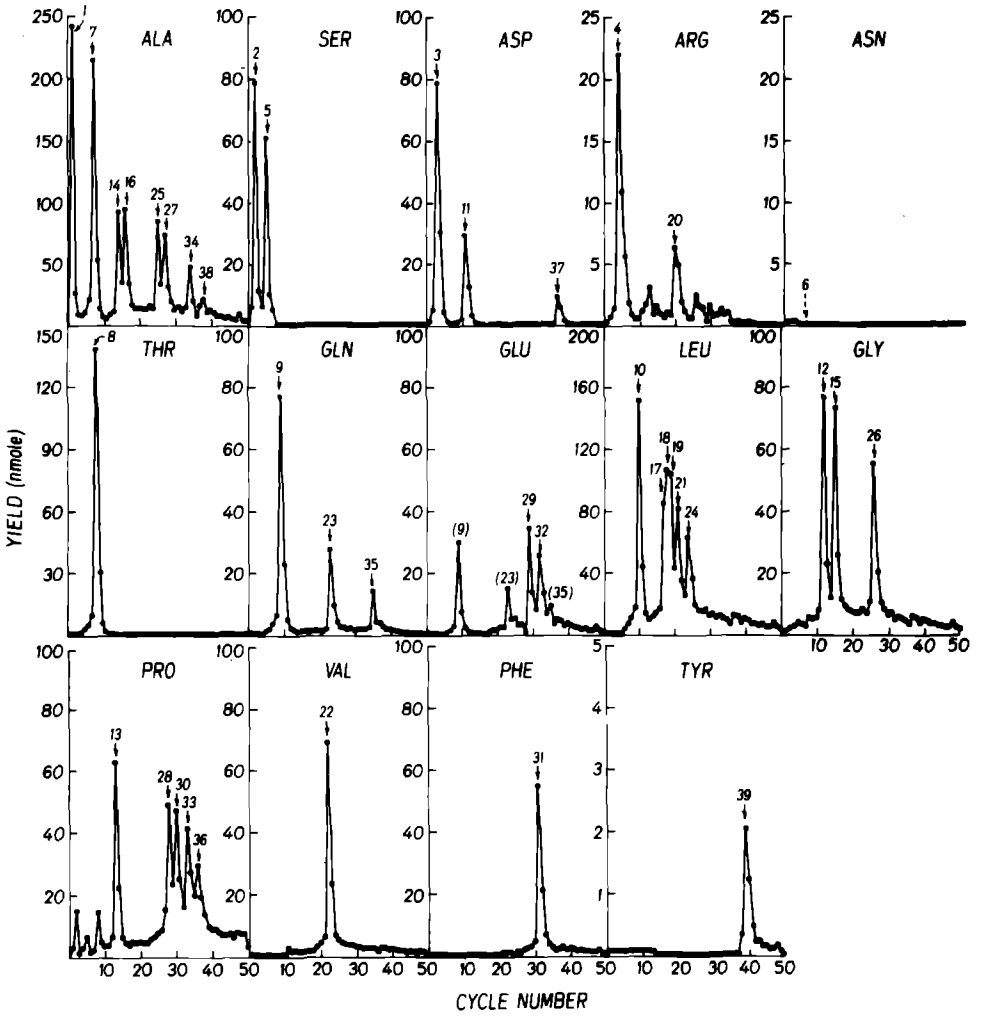

Fig. 2. Yields of each PTH-amino acid obtained during the sequence of the HPGP peptide isolated, as a function of sequenator cycle number. The numbers above each peak denote the assigned sequence position of that particular residue. In the case of PTH-Glu, numbers in parenthesis denote the assignement of that residue as a PTH-Gln, since partial deamidation occurs during the PTH-conversion procedure (1.5N HCl/Methanol, 65°C). At cycle 6, no residue could be identified, and it is proposed to be a glycosylated Asn residue (see text).

performed. Then 1 mg of the human glycopeptide was added and a double coupling done for the first cycle only. The sequence was extended to 50 cycles without any further modifications. All conversions of the phenylthiazolinones to phenylthiohydantoins (PTH) were done automatically immediately following the cleavage step using 1.5 N HCl/MeOH at 65°C. This allowed good recoveries for PTH-Ser and PTH-Thr (see Fig. 2). The PTH-amino acids were separated by HPLC on an Altex 5μ-ultrasphere ODS (0.46 x 25 cm) as previously reported (7) except that PTH-Glu and PTH-Asp were detected as their respective methyl esters, PTH-NorLeu was used as an internal standard, and detection made at 269 nm.

Table 1

Amino acid analysis following 24, 48 and 72 hrs acid hydrolysis of the isolated HPGP. Each value represents the mean of triplicate analysis. The amounts of glucosamine (GlcN) and galactosamine (GalN) were not corrected for hydrolysis losses due to their destruction.

Amino Acid	24 hrs	48 hrs	72 hrs	Integer Values
Asx	3.88	3.88	3.78	4
Thr	1.00	0.96	0.93	1
Ser	1.91	1.73	1.57	2
Glx	5.39	5.40	5.39	5
Pro	5.49	5.45	5.46	5
Gly	3.03	2.96	2.94	3
Ala	7.68	7.68	7.66	8
Val	0.73	0.73	0.71	1
Leu	6.01	6.00	6.00	6
Tyr	0.19	0.18	0.18	1*
Phe	0.94	0.93	0.93	1
Arg	2.00	2.11	2.03	2
GlcN	0.88	0.55	0.27	+++
GalN	0.12	0.04	0.02	+
Presumed Total				39

* The Tyr value is low even in presence of phenol in the hydrolysis tube. Therefore although Tyr carboxy-terminal was found (see Fig. 2, 3), the amino acid composition data indicate that about 80% of the isolated molecules lost their C-terminal Tyr.

(d) Polyacrylamide/SDS slab gel electrophoresis

The polyacrylamide slab gel electrophoresis contained 15% acrylamide and was performed according to Laemmli (8). The molecular weight markers used varied between 94,000 and 14,000 daltons (Pharmacia Fine Chemicals).

Results

In Fig. 1A, the initial fractionation of the human pituitary extract (unretained peak on CM-cellulose) by HPLC is depicted. The peak denoted HNT represents the human N-terminal segment of pro-opiomelanocortin previously reported (1,2). The peak denoted HPGP (human pituitary glycopeptide) was repurified under the same conditions as shown in Fig. 1B and provided the peptide on which sequence was performed. Furthermore on SDS slab gels this peptide migrated as a single band with an apparent molecular weight of 12,800.

In Table 1, the results of the amino acid analysis of this peptide are shown. It is seen that based on these data the length of the peptide is presumed to be 39 amino acids. The low Tyr value indicates that about 80% of the isolated peptide lacks a Tyr residue. Furthermore, the presence of glucosamine (GlcN) and some galactosamine (GalN) indicated that this peptide is glycosylated.

The yields of each PTH-amino acid during the sequence of this peptide are shown in Fig. 2. It can be seen that at cycle 6 no residue could be identified. This sequence proceeded directly to the C-terminal Tyr residue. It is, however, noted that the yield of the C-terminal Tyr is low as was expected from the

Fig. 3. Proposed complete sequence of the isolated human pituitary glycopeptide. A comparison with the sequences reported for the homologous pig, sheep and ox peptides (4) is also shown. Residues that differ from the human structure are boxed in.

amino acid composition (Table 1). Based on these sequence results (Fig. 2) and the amino acid composition (Table 1), it can be seen that 38 out of the 39 residues can be accounted for by the sequence, with the exception of one Asx residue. Since this peptide is glycosylated, the presence of the characteristic triad Asx_6-X-Thr_8 would place the missing Asx as a glycosylated Asn at residue 6. This conclusion is supported by the absence of an identifyable residue at cycle 6, which can be explained by the insolubility of glycosylated Asn in the butylchloride sequencer solvent (2,9). Furthermore, this would fit with the rule that glycosylated Asn is always present in a characteristic Asn-X-Thr(Ser) sequence (10). Also, Asn-linked oligosaccharides are very rich in glucosamine (11) and could contain minor amounts of galactosamine (12). This is exactly what was observed in Table 1, where the ratio of GlcN to GalN (uncorrected for hydrolysis loss) is large. Accordingly, the proposed sequence of this 39 amino acids human glycopeptide is shown in Fig. 3. Here a comparison is also made with that reported for homologous peptides obtained from pig (3,4), ox (4) and sheep (4) pituitaries.

Discussion

As seen from Fig. 3, only 5 to 6 variations out of 39 are observed between the sequence of this human peptide and those of ox, sheep and pig homologues (4). Furthermore, all the homologous peptides isolated have a maximum length of 39 residues and are glycosylated at Asn-6. Although a shorter peptide was first isolated (residues 1-17) from pig posterior pituitaries (3), its presence in this tissue would suggest that it could be synthesized in the hypothalamus, rather than the pituitary. If so, it would then be transported and stored in the neurohypophysis (13). In this tissue few hormones are known to be present, namely,

the neurophysins (NP) and their associated arginine vasopressin (AVP) and oxytocin (OT) (14), pro-enkephalins (15,16), pro-somatostatin (17,18) and pro-cholecystokinin (19). Recently the biosynthesis of AVP and OT indicated that these two hormones are synthesized via two separate precursors containing two neurophysins (13,20,21). Different from the NP-OT precursor, the NP-AVP one contains an "N-terminal" N-glycosylated extension (20). Based on the reported apparent molecular weights (Mr) on SDS [20,000 (13) to 23,000 (20)] and those known for neurophysins (about 10,000) and AVP (about 1000), the expected Mr on SDS for the glycopeptide extension for pro-NP-AVP would vary between 9,000 and 12,000.

From the data presented in this paper, and the above considerations, the 39 residues glycopeptide isolated may be part of the missing "N-terminal" extension of pro-NP-AVP, since its Mr on SDS is 12,800 and a similar glycopeptide is found in the posterior lobe of porcine pituitaries (N. Larivière, unpublished data). A similar abnormal migration on SDS (Mr 13,000) has also been observed for the glycosylated 39 residues mouse ACTH (22). Furthermore, since in all species studied, Tyr-39 occupies the C-terminal residue, it would not be surprising if in the precursor form of this molecule, a pair of basic residues would follow the Tyr moiety, similar to what is known about pro-secretory proteins (2,23-25). However, this glycopeptide could also form part of another precursor molecule synthesized in the hypothalamus, pro-somatostatin (17,18) and pro-opiomelanocortin (1,2) now being excluded. It is therefore necessary to delienate between these various possibilities as to the origin and functional role of this highly conserved glycopeptide.

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References

1. Seidah, N.G., Benjannet, S., Routhier, R., De Serres, G., Rochemont, J., Lis, M. and Chrétien, M. (1980) *Biochem. Biophys. Res. Commun.* 95, 1417-1424.
2. Seidah, N.G. J. Rochemont, J. Hamelin, M. Lis and Chrétien, M. (1981) J.Biol. Chem. (In Press).
3. Holwerda, D.A. (1972) Eur. J. Biochem. 28, 340-346.
4. Smyth, D.G. and Massey, D.E. (1979) Biochem. Biophys. Res. Commun. 87, 1006-1010.
5. Chrétien, M., Benjannet, S., Dragon, N., Seidah, N.G. and Lis, M. (1976) Biochem. Biophys. Res. Commun. 72, 472-478.
6. Fauconnet, M. and Rochemont, J. (1978) Anal. Biochem. 91, 403-409.
7. Somack, R. (1980) Anal. Biochem. 104, 464-468.
8. Laemmli, U.K. (1970) Nature 227:680-685.
9. Thomas, K.A., Baglan, N.C. and Bradshaw, R.A. (1981) J. Biol. Chem. (in press).
10. Pless, D.D. and Lennarz, W.J. (1977) Proc. Natl. Acad. Sci. USA 74, 134-138.

11. Li, E., Tabas, I. and Kornfeld, S. (1978) J. Biol. Chem. 253, 7762-7770.
12. Bahl, O.P., Reddy, M.S. and Bedi, G.S. (1980) Biochem. Biophys. Res. Commun. 96, 1192-1199.
13. Brownstein, M.J., Russell, J.T. and Gainer, H. (1980) Science 207, 373-378.
14. Sachs, H. (1969) Neurosecretion. In: "Advances in Enzymology and Related Areas of Molecular Biology", vol. 32, FF. Nord, ed., Interscience, New York, pp. 1-78.
15. Goldstein, A. and Ghazarossian, V.E. (1980) Proc. Natl. Acad. Sci. USA 77, 6207-6210.
16. Martin, R. and Voigt, K.H. (1981) Nature 289, 502-504.
17. Hobart, P., Crawford, R., Shen, L., Pictet, R. and Rutter, W.J. (1980) Nature 288, 137-141.
18. Goodman, R.H., Jacobs, J.W., Chin, W.W., Lund, P.K., Dee, P.C. and Habener, J.T. (1980) Proc. Natl. Acad. Sci. USA 77:5869-5873.
19. Beinfeld, M.C., Meyer, D.K. and Brownstein, M.J. (1980) Nature 288, 376-378.
20. Richter, D., Schmale, H., Ivell, R. and Schmidt, C. (1980) In: "Biosynthesis, Modification and Processing of Cellular and Viral Polyproteins", G. Koch and D. Richter, eds., Acad. Press, New York, pp. 43-66.
21. Cohen, P., Nicolas, P., Lauber, M., Masse, M.J.O., Boussetta, H., Beguin, P., Camier, M. (1980) In: "Biosynthesis, Modification and Processing of Cellular and Viral Polyproteins", G. Koch and D. Richter, eds., Acad. Press, New York, pp. 15-28.
22. Eipper, B.A., Mains, R.E. and Guenzi, D. (1976) J. Biol. Chem. 251, 4121-4126.
23. Steiner, D., Quinn, P.S., Chan, S.J., Marsh, J. and Tager, H.S. (1980) Ann. N.Y. Acad. Sci. 343, 1-16.
24. Chrétien, M., Benjannet, S., Gossard, F., Gianoulakis, C., Crine, P., Lis, M. and Seidah, N.G. (1979) Can. J. Biochem. 57:1111-1121.
25. Chrétien, M. and Seidah, N.G. (1981) Mol. & Cell. Biochemistry 34, 101-127.