The complete amino-acid sequence of anglerfish somatostatin-28 II

A new octacosapeptide containing the (Tyr^7, Gly^{10}) derivative of somatostation-14 I*

Alain Morel[1], Jui-Yoa Chang[2] and Paul Cohen[1]

[1]*Groupe de Neurobiochimie Cellulaire et Moléculaire, Unité Associée au CNRS N°. 554, Université Pierre et Marie Curie, 96 boulevard Raspail. 75006 Paris, France,* and [2]*Ciba-Geigy Ltd., CH-4002 Basel, Switzerland*

Received 19 July 1984

A new somatostatin-28 has been isolated from the Teleostean fish *(Lophius piscatorius)* Brockmann organs. Determination of its aminoacid sequence indicates that it corresponds to an octacosapeptide containing in its C-terminal end the Tyr-7 Gly-10 derivative of somatostatin-14 I. This structure is in agreement with the one predicted by Hobart et al. (Nature (1980) 288, 137–141) from a cDNA nucleotide sequence. It demonstrates that, since the corresponding somatostatin-14 II cannot be detected in this organ, S-28 II is a terminal product of prosomatostatin II processing in anglerfish pancreatic islets.

Amino-acid sequencing *Anglerfish* *Somatostatin-28 II* *Somatostatin-14 I* *Pancreatic islet*

1. INTRODUCTION

The Brockmann organ from the teleostean fish *Lophius piscatorius* is an endocrine pancreas tissue [3] containing large amounts of somatostatin material [4]. Expression of gene coding for this peptide has been the subject of several investigations [5,6]. In 1980, Rutter and his collaborators cloned two cDNA corresponding to two mRNA species from this organ [7]. The nucleotide sequences of these DNA indicated that one of the two polymers encodes a prosomatostatin containing, at its C-terminal end, a new tetradecapeptide called somatostatin-14 II [7]. This sequence corresponds to the Tyr-7, Gly-10 derivative of the somatostatin-14 I found in the brain and in pancreatic tissues [8]. Attempts to detect this second variety of somatostatin-14 both in neural and pancreatic tissues remained unsucessful [9,10]. However, results by others indicated that this tetradecapeptide could be included into a larger form deriving from the corresponding precursor, prosomatostatin II [11]. We have recently shown that anglerfish Brockmann organs contain a single species of S-14 indistinguishable from S-14 I of both hypothalamic and pancreatic tissues [4]. In contrast, analysis by HPLC of the S-28 forms indicated that two distinct forms could be separated [4]. Both immunochemical and biochemical evidences [4] have shown that one of the two species could correspond to S-28 II, the C-terminal octacosapeptide fragment of prosomatostatin II, including the Tyr-7, Gly-10 derivative of somatostatin-14 I. Moreover, exposure of this new somatostatin-28 to an Arg-Lys-esteropeptidase preparation from the

Abbreviations: DABSCI, dimethylaminoazobenzene sulphonyl chloride; DABITC, 4-*N,N*-dimethylaminobenzene 4′isothiocyanate; PITC, phenylisothiocyanate; HPLC, High Performance Liquid Chromatography; PTH, phenylthiohydantoin

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

rat brain cortex [12] led to quantitative conversion into a tetradecapeptide identified as somatostatin-14 II by HPLC [4].

In the present report we demonstrate that this hypothesis is indeed correct by determining the complete amino acid sequence of the isolated peptide.

2. MATERIALS AND METHODS

Somatostatin-28 II was extracted from anglerfish pancreatic islets freshly collected from live animals on the fishing boat (thanks to the kind hospitality of Captain Riou and his crew, La Marie-Sophie, Le Conquet, Finistère, France). This peptide was purified by successive HPLC steps using a reverse phase 10RP8 Lichrosorb column (Merck) eluted by a mixture of acetonitrile/trifluoroacetic acid 1‰ (v/v) in H_2O/trifluoroacetic acid 1‰ (v/v). A linear gradient from 28 to 33% acetonitrile was employed at 1 ml · min^{-1} for 30 min. This operation was repeated, using the same methodology, until complete separation of somatostatin-28 II was achieved. The HPLC system consisted in a Spectra Physics (SP 8000) system, and a Kratos SF740 uv detector (Schoeffel) was used for monitoring peptides in the column effluent.

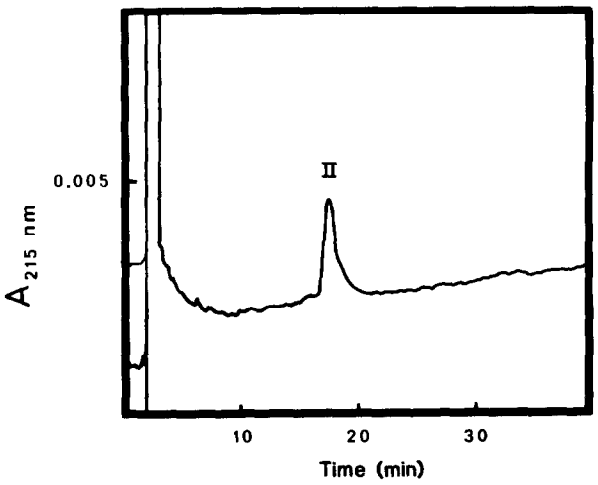


Fig. 1. Purification by HPLC of somatostatin-28 II isolated from anglerfish Brockmann organs. The S-28 species recovered from a Sephadex G-50 column fractionation [4] were first submitted to reverse-phase HPLC on a 10RP8 column (4 × 250 mm) eluted with a mixture of acetonitrile/trifluoroacetic acid 1‰ (v/v) in H_2O/trifluoroacetic acid 1‰ (v/v). A linear gradient from 28 to 33% acetonitrile was employed at 1 ml · min^{-1} for 30 min. This operation was repeated with the same methodology. A typical result is shown. The trace represents elution of the isolated S-28 II previously separated from S-28 I, monitored at 215 nm as a function of the retention time.

N-terminal analysis was performed by the manual double coupling method of Chang [13] using DABITC/PITC (from Fluka, Switzerland). The amino acid composition was determined on a hydrolysate of the peptide after derivatization of the released residues by DABSC1 ([14]; Fluka) followed by HPLC analysis of the derivatives [15]. Fragmentation was accomplished on 1 nmol of the peptide, using endolysine as protease (Boehringer, FRG). Automatic sequencing was carried out by a gas-phase U70A protein sequanator using program Q provided by Applied Biosystems [16]. PTH amino acids were then analyzed by HPLC according to [17].

3. RESULTS AND DISCUSSION

The isolated peptide was found homogeneous by HPLC criteria (see fig. 1) and N-terminal amino

Table 1

Amino acid composition of anglerfish Somatostatin-28 II

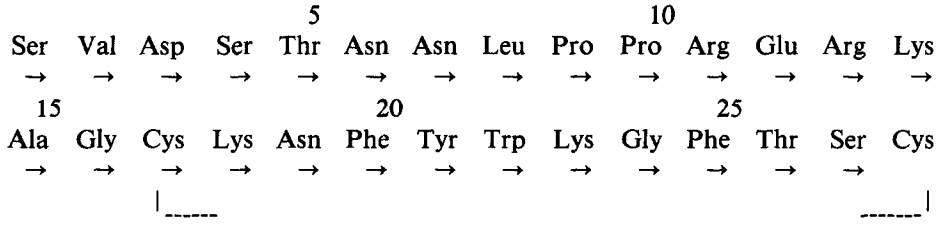
Amino acid	Found residues per mol	Theoretical
Asp	3.7	4
Glu	1.3	1
Ser	3	3
Thr	1.8	2
Gly	1.8	2
Ala	0.9	1
Arg	1.7	2
Pro	1.7	2
Val	0.7	1
Leu	1.2	1
Phe	2	2
Cys	n.d.	2
Lys	2.6	3
Tyr	1.3	1
Trp	n.d.	1

n.d., not determined.

acids analysis. The amino acid composition, determined on an hydrolysate using the HPLC separation of the DABSCl derivatives [15], was in good agreement with the theoretical one (table 1). It showed in particular the presence of a tyrosine and of an extra glycine residue. The complete sequence was determined using the following strategy. Firstly, the intact peptide was analyzed by the manual double-coupling method [13] and the residues Ser^1, Val^2, Asp^3, Ser^4 and Thr^5 were determined. Secondly, the peptide was digested with endolysine which generates four fragments corresponding to cleavage at Lys^{14}, Lys^{18}, Lys^{23} and the fragments were analyzed for their N-terminal end by three successive cycles of the Chang double coupling method [13]. Ser^1, Ala^{15}, Asn^{19}, Gly^{24} then Val^2, Gly^{16}, Phe^{20} and Phe^{25}, then Asp^3, Tyr^{21} and Thr^{26} were found in the successive cycles.

Third, the complete amino acid sequence was determined by automated Edman degradation followed by HPLC analysis of the PTH derivatives.

The deduced sequence is the following:



As can be observed Tyr^{21} and Gly^{24} substitute the usual Phe^{21} and Thr^{24} of the somatostatins 28 from both mammalian pancreas and hypothalamus [18–20].

Much wider discrepancies were found in the N-terminal half of the molecule (1→12 portion) upstream from the Arg^{15} Lys^{16} doublet. Substitutions at positions 2,3,5,6,7,8,9 were found when compared to the mammalian octacosapeptide [18–20]. Since this new peptide exhibits, in an heterologous system [4], a strong biological potency (inhibition of growth hormone release by rat anterior pituitary cells), this raises interesting questions about the putative biological role of the S-28 II (1→12) fragment. The fact that in vivo the octacosapeptide does not generate the corresponding S-14 II terminal fragment does not exclude that this 1–12 moiety either plays a role in the overall conformation of the octacosapeptide or else as such if released. It provides also suggestive, but indirect evidence in favor of a processing of prosomatostatin via the S-28 form. That this latter octacosapeptide is not simply an intermediate in the prosomatostatin →S-14 conversion and does not work simply as a precursor of the tetradecapeptide is suggested by direct binding studies [21].

The exact biological function of this somatostatin-28 II remains to be discovered in the gastrointestinal tract of the anglerfish.

ACKNOWLEDGEMENTS

This work was supported in part by funds from the Université Pierre et Marie Curie, the Centre National de la Recherche Scientifique (UA No. 554), the Fondation pour la Recherche Médicale Française and the Institut National de la Santé et de la Recherche Médicale (INSERM, CRE No. 834006).

REFERENCES

[1] Cohen, P., Nicolas, P., Lauber, M., Morel, A., Gluschankof, P., Gomez, S. and Camier, M. (1984) in: 4ème Ecole Franco-Africaine de Biologie Moléculaire, D'Jerba, (F. Ben Hamida, ed.).

[2] Cohen, P., Morel, A., Gluschankof, P., Gomez, S. and Nicolas, P. (1984) in: Somatostatin (Y.C. Patel and G. Tannenbaum, eds.), Plenum Press, New York.

[3] Rennie, J. (1905) Q. J. Microsc. Sci. 48, 379–404.

[4] Morel, A., Gluschankof, P., Gomez, S., Fafeur, V. and Cohen, P. (1984) Proc. Natl. Acad. Sci. USA, in press.

[5] Goodman, R.H., Lund, P.K., Jacobs, J.W. and Habener, J.F. (1980) J. Biol. Chem. 255, 6549–6552.

[6] Shields, D. (1980) J. Biol. Chem. 255, 11625–11628.

[7] Hobart,P., Crawford, R., Shen, L.P., Pictet, R. and Rutter, W.J. (1980) Nature 288, 137–141.

[8] Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. and Guillemin, R. (1973) Science 179, 77–79.
[9] Morel, A., Nicolas, P. and Cohen, P. (1983) J. Biol. Chem. 258, 8273–8276.
[10] Noe, B.D. (1983) J. Biol. Chem. 256, 9397–9400.
[11] Noe, B.D. and Spiess, J. (1983) J. Biol. Chem. 258, 112i–1128.
[12] Gluschankof, P., Morel, A., Gomez, S., Nicolas, P., Fahy, C. and Cohen, P. (1984) Proc. Natl. Acad. Sci. USA, in press.
[13] Chang, J.Y., Brauer, D. and Wittmann-Liebold, B. (1978) FEBS Lett. 93, 205–214.
[14] Chang, J.Y., Knecht, R. and Braun, D.G. (1981) Biochem. J. 199, 547–555.
[15] Chang, J.Y., Knecht, R. and Braun, D.G. (1982) Biochem. J. 203, 803–806.
[16] Hewick, R.M., Hunkapiller, M.W., Hood, L.E. and Drieyer, W.J. (1983) J. Biol. Chem. 256, 7990–7997.
[17] Knecht, R., Seermüller, V., Liersch, M., Fritz, H., Braun, D. and Chang, J.Y. (1983) Anal. Biochem. 130, 65–71.
[18] Pradayrol, L., Jornvall, H., Mutt, V. and Ribet, A. (1980) FEBS Lett. 109, 55–58.
[19] Esch, F., Bolhen, P., Ling, N., Benoit, R., Brazeau, P. and Guillemin, R. (1980) Proc. Natl. Acad. Sci. USA 77, 6827–6831.
[20] Spiess, J., Villareal, J. and Vale, W. (1981) Biochemistry 20, 1982–1988.
[21] Srikant, C.B. and Patel, Y.C. (1982) Nature 294, 259–260.