STRUCTURAL STUDIES ON OVINE GROWTH HORMONE. SEQUENCE OF A CYANOGEN BROMIDE FRAGMENT

H.N. FERNÁNDEZ, A.C. PALADINI, J.M. DELLACHA and J.A. SANTOMÉ

Facultad de Farmacia y Bioquímica, Departamento de Química Biológica y Centro para el Estudio de las Hormonas Hipofisarias, Junín 956, Buenos Aires, Argentina

Received 8 July 1971

1. Introduction

Several physicochemical parameters indicate the existence of a great analogy between ovine (OGH) and bovine growth hormone (BGH) molecules, as reviewed in a previous paper [1] in which the following structural analogies were also substantiated: identical amino acid sequence in their *N*-terminal peptides; identical sequence in the last thirteen amino acids of the chain; similar distribution of methionyl residues along the polypeptide chain and almost identical amino acid composition of cyanogen bromide fragments 1 and 2 [1] in OGH with the segments of BGH occurring between methionines 5–121, 146–176 and 121–146 [2], respectively.

In the present paper we give the experimental data supporting the total sequence of cyanogen bromide fragment 2 (CNBr-2) in OGH.

2. Material and methods

These were as indicated in [1] and [3] unless stated otherwise. Tryptic cleavages were done in a pH-stat at pH 8.6 with a 1:66 weight ratio of enzyme to peptide. Peptic digestions were carried out during 16 hr at 37° in 5% formic acid with a 1:40 weight ratio of enzyme (Pepsin, 2× crystallized, Worthington Biochemicals) to substrate. Leucine aminopeptidase (Sigma) was used as indicated by Schroeder and Roger [4]. Partial acid hydrolysis was performed following the Tsung and Fraenkel-Conrat [5] instructions.

The chromatographic fractionation of peptide mixtures was done in a column 0.6 cm × 20 cm of a sulfonic resin (Chromobeads Type C II, Technicon Corp., New York) equilibrated and eluted at 60°, with a 0.2 M pyridine acetate buffer pH 3.1, increasing linearly its pH and concentration to 5.0 and 2.0, respectively. Elution rates were usually between 15 ml and 24 ml per hr.

3. Results and discussion

Fig. 1a shows the result of the chromatographic fractionation of a tryptic digest of CNBr-2, and table 1 indicates the amino acid composition of the peptides thus isolated, together with that of the starting peptide.

Fig. 1b shows the result of the chromatographic fractionation of T_3 partially hydrolyzed with acid; T_3H_1 is free aspartic acid. The amino acid compositions and Edman degradations recorded in table 2 established the total sequence of T_3H_3 and the partial sequence of T_3H_2.

On incubation of T_3 with leucine aminopeptidase the enzymatic action stopped after the liberation of the following amino acids, expressed in moles per mole of peptide: Arg: not determined, Glu: 1.84 and Leu 0.84. These results identified peptide T_3H_3 as *N*-terminal in T_3. The localization of T_3H_2 at the other end of T_3, with *C*-terminal arginine, as well as the placement of one aspartyl residue in a middle position, joining both tetrapeptides, becomes the only

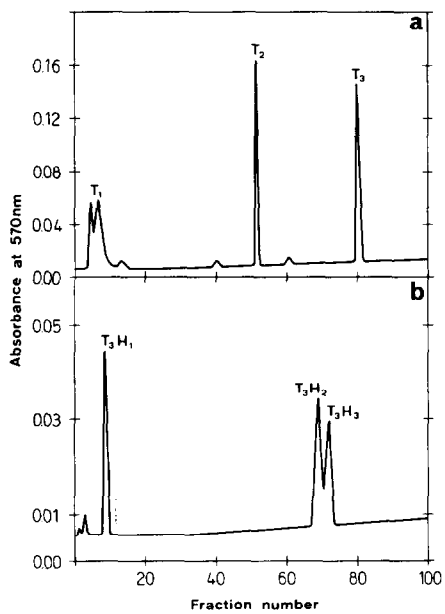

Fig. 1. Chromatographic fractionation of the peptides arising from: (a) tryptic hydrolysis of CNBr-2; (b) partial acid hydrolysis of peptide T_3. (See Materials and methods for details).

alternative to explain all the following experimental facts: T_3 is a tryptic peptide, hence should have a basic amino acid in the *C*-terminal end; acid hydrolysis of T_3 liberates aspartic acid and two tetrapeptides (fig. 1b); the amino acid composition of T_3H_2 plus that of T_3H_3 and an estimated amount of 1 mole of aspartic acid, account for the total composition of T_3 (tables 1 and 2); the detection of leucine aminopeptidase action after degrading the *N*-terminal tetrapeptide in T_3 is in line, according to its specificity [4], with the existence of an aspartyl residue in the next position, as postulated above. The negative charge shown by T_3 on paper electrophoresis at pH 6.5, supports this conclusion. The sequence of T_3 is then established as: Arg–Glu–Leu–Glu–Asp–Val–Thr–Pro–Arg.

Peptide T_2 incubated 3.5 hr with a mixture of carboxypeptidases A plus B liberated the following amino acids, expressed in moles per mole of peptide: Lys: 1.00; Leu: 0.69; Ile: 0.65 and Gln: 0.27. A short incubation of 30 min with carboxypeptidase B gave: Lys: 1.00; Leu: 0.45 and Ile: 0.34. These results established the partial sequence: (Ala, Gly) Gln–Ile–Leu–Lys for T_2. Two steps of Edman degradation gave the relative positions of the remaining

Table 1
Amino acid composition of CNBr-2 and of the peptides arising from its tryptic cleavage.

Amino acid	Peptide			
	CNBr-2	T_1	T_2	T_3
Arg	0.87			0.82
Glx	0.95			0.90
Leu	0.92			1.06
Glx	0.95			0.90
Asx	0.96			0.97
Val	1.10			0.94
Thr	1.02			1.03
Pro	1.33			1.00
Arg	0.87			0.82
Ala	1.20		0.93	
Gly	1.10		0.93	
Glx	0.95		1.13	
Ile	0.87		1.04	
Leu	0.92		1.06	
Lys	0.90		0.93	
Glx	0.95	1.04		
Thr	1.02	1.12		
Tyr	0.90	0.77		
Asx	0.96	0.95		
Lys	0.90	0.86		
Phe	1.09	0.91		
Asx	0.96	0.95		
Thr	1.02	1.12		
Asx	0.96	0.95		
Hser	n.d.	0.35		

Values are expressed as moles per mole of peptide. The amino acids are tabulated in descending order, starting from the *N*-terminus and following their correct sequence in each peptide; to those occurring more than once the corresponding fraction of the total value found in the analysis was assigned to each position.
n.d.: not determined.

amino acids. The composition of the residual peptide from T_2 after the first step was: Ala: 0; Gly: 1.05; Glu: 1.17; Ile: 1.08; Leu: 1.03 and Lys: 0.70; the second step gave: Ala: 0; Gly: 0; Glu: 1.10; Ile: 1.02; Leu: 1.10 and Lys: 0.77. The sequence of T_2 was thus established as: Ala–Gly–Gln–Ile–Leu–Lys.

The 5 Edman degradations of peptide T_1 showed in table 3 established the following sequence: Glx–Thr–Tyr–Asx–Lys– (Phe, Asx, Thr, Asx, Met). Peptide T_1 must be *C*-terminal in CNBr-2 since it is the only fragment containing homoserine. This conclusion is strengthened by the result of a carboxypep-

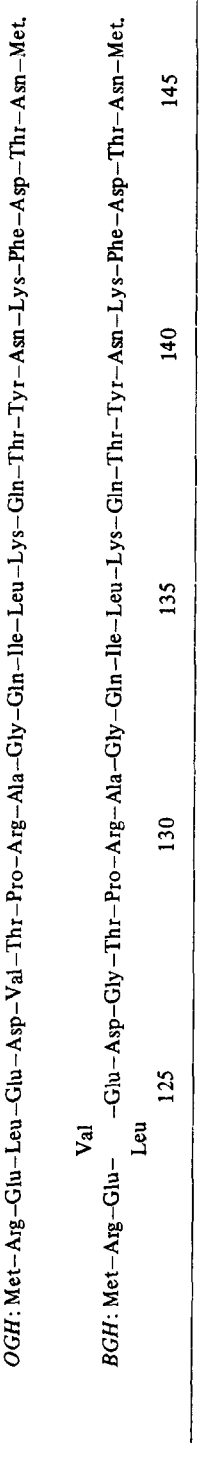

Fig. 2. Homology of the region in the OGH molecule giving rise to CNBr-2 with the peptide chain in BGH between methionines 121 and 146.

Table 2
Edman degradation of peptides T_3H_2 and T_3H_3.

Step	Amino acids (subtractive method)							
	T_3H_3				T_3H_2			
	Arg	Glx	Leu	Glx	Val	Thr	Pro	Arg
0	0.98	1.00	1.20	1.00	0.89	1.12	0.93	1.08
1	*0*	0.97	1.06	0.97	*0.15*	0.95	0.81	1.20
2	0	*0.37*	1.00	1.00	0.14	*0.41*	1.03	0.97
3	0	0.37	*0.59*	1.00				

Values are expressed in moles per mole of peptide. The amino acids are presented in their correct sequence. Half of the total value found in the analysis was assigned to each glutamic acid in T_3H_3. The italicized values refer to the amino assumed to be lost at the indicated degradation step.

Table 3
Edman degradation of peptide T_1.

Step	Amino acids (subtractive method)									
	Glx	Thr	Tyr	Asx	Lys	Phe	Asx	Thr	Asx	Hser
0	1.04	1.12	0.77	0.95	0.86	0.91	0.95	1.12	0.95	0.35
1	*0.52*	1.06	0.91	1.08	0.91	0.95	1.08	1.06	1.08	n.d..
2	0.52	*0.62*	0.83	1.05	0.94	1.08	1.05	1.00	1.05	n.d.
3	0.42	0.72	*0.27*	1.03	0.92	1.00	1.03	1.00	1.03	n.d.
4	0.73	0.75	0.33	*0.75*	0.88	1.13	1.00	1.00	1.00	n.d.
5	0.69	0.82	0.28	0.96	*0.48*	1.04	1.00	1.00	1.00	n.d.

Values are expressed as moles per mole of peptide. The amino acids are tabulated starting from the *N*-terminus and following their correct sequence in the peptide. To those occurring more than once, the corresponding fraction of the total value found in the analysis was assigned to each position. The italicized values refer to the amino acid assumed to be lost at the indicated degradation step. n.d.: not determined.

tidase A incubation of CNBr-2 which released, per mole of peptide, 0.93 mole of homoserine and 1.04 mole of asparagine or threonine or, of a mixture of both compounds. This uncertainty was due to the fact that the particular program of elution applied on this run of the analyzer was not able to resolve these two amino acids into separate peaks. No aspartic acid was detected.

Incubation of CNBr-2 with pepsin gave rise to eight peptides well resolved by the chromatographic procedure described in Materials and methods. Peptic fragments number 3 (P_3) and 8 (P_8) had the amino acid

Table 4
Amino acid composition of peptides P_3 and P_8 arising from CNBr-2 by peptic hydrolysis and Edman degradation of P_3.

Step	Amino acids (subtractive method)										
	P_8							P_3			
	Lys	Glx	Thr	Tyr	Asx	Lys	Phe	Asx	Thr	Asx	Hser
0	0.94	0.98	1.11	1.04	0.89	0.94	1.01	0.92	1.18	0.92	0.66
1								*0*	1.05	0.96	0.63
2								0	*0.23*	1.00	0.61
3								0	0.19	*0.31*	0.61

Values are expressed in moles per mole of peptide. The amino acids are presented in their correct sequences. Half of the total value found in the analysis was assigned to each glutamic acid in T_3H_3. The italicized values refer to the amino acid assumed to be lost at the indicated degradation step.

composition indicated in table 4 where is also recorded the Edman degradation of P_3.

The amino acid compositions of P_8 and P_3 account for the composition of peptide T_1 plus an extra lysyl residue (located in P_8). The presence of *C*-terminal homoserine in P_3 justifies the relative positions assigned to P_3 and P_8 in table 4, and the extra lysyl residue in P_8 must coincide with the *N*-terminal lysine in T_2, thus joining T_1 and T_2 in their proper order.

Incubation of P_8 with leucine aminopeptidase liberated the following amino acids, expressed in moles per mole of peptide: Lys: not determined; Gln: 1.0; Thr: 1.0; Tyr: 0.61; Asn: 0.61 and Phe: 0.61.

At this stage of the structural study of CNBr-2 there remains only one position left in which to place T_3 in the molecule, that is by joining its *C*-terminal end with the *N*-terminal end of T_2. The complete sequence of CNBr-2 is thus established.

The state of amidation of the aspartyl residue in P_3, not yet clarified, could be deduced by the behaviour of CNBr-2 during paper electrophoresis at pH 6.5 where it proved to be neutral: this balance of electrical charges can only be reached if the β-carboxyl of that amino acid is free.

In fig. 2 is shown the primary structure of the region in OGH giving rise to CNBr-2 compared to its homologous zone in BGH [2]. There are only two differences between both hormones: glycine 127 in BGH is replaced by valine in OGH, and the microheterogeneity displayed by BGH in position 124 [6, 7] is not present in OGH.

Acknowledgements

We are indebted to Miss Dora M. Beatti, Miss Nélida Ramos and Mr. O.A. Duffort for excellent technical assistance. H.N. Fernández is a fellow of the Instituto Nacional de Farmacología y Bromatología.

A.C. Paladini, J.M. Dellacha and J.A. Santomé, are Career Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina. This work has been supported in part, by grants from the same institution.

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