STRUCTURAL STUDIES ON OVINE GROWTH HORMONE

Complete amino acid sequence, partially based on homology with bovine growth hormone

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1. Introduction

Physicochemical data supporting the great analogy between ovine and bovine growth hormones has been previously reviewed [1]. The close homology in their primary structures was first indicated by very similar peptide maps [2] which were subsequently used by Seavey et al. [3] to pinpoint the only differences between them as located in a single tryptic peptide. The complete sequence of a cyanogen bromide fragment in ovine growth hormone, reported by Fernández et al. [4], was found identical to the corresponding fragment in bovine growth hormone, except for two replacements which were the same as those occurring in the tryptic peptide of Seavey et al. [3]. Other analogies between ovine and bovine growth hormones are: identical amino acid sequence and microheterogeneity in their N-terminal peptides; identical sequence of the last 13 amino acids in the chain and similar distribution of the methionyl residues in the molecule [1].

The chemical study of ovine growth hormone has reached a point in our laboratory where it is possible to express its complete amino acid sequence by a unique combination of tryptic, chymotryptic and peptic peptides, plus information mentioned before [1,2,4]. The amino acid sequence of a number of peptides is based on homology with bovine growth hormone.

2. Materials and methods

These were as indicated in [1,2,4], unless stated otherwise. The compositions of the solvents used for paper chromatography were as follows, solvent 1: butan-1-ol–acetic acid–water (4:1:5, by vol); solvent 2: butan-1-ol–acetic acid–water–pyridine (5:1:4:3.3, by vol).

2.1. *Tryptic peptide maps*

Digestions were carried out with native or oxidized hormone. The chromatography was performed for 18 hr with solvent 1 and the electrophoresis lasted 1 hr at 50 V/cm. The peptide spots, located by reaction with ninhydrin, were eluted with 5.7 M hydrochloric acid or 5% acetic acid and the solutions used for amino acid composition or N-terminal determinations, respectively.

2.2. *Isolation of tryptophan-containing peptides*

The citraconylated [6] hormone was digested with trypsin and then submitted to electrophoresis at pH 6.45 for 1 hr, at 50 V/cm. The band in which the digest was originally applied was cut and stitched to a sheet of Whatman 3MM paper. By descending chromatography during 24 hr with solvent 1, it was possible to isolate in pure condition peptide TC_8 (table 1).

Another tryptophan-containing peptide was isolated by the following procedure: a tryptic digest of native hormone dissolved in 20% formic acid was filtered through a Sephadex G-50 column (2.6 × 90 cm), and eluted at 15 ml per hour with the same solvent. The Ehrlich-positive material appearing in the void volume was collected and digested again with trypsin in the presence of 0.1% sodium dodecyl sulphate. From this second digest it was possible to isolate in

Table 1
Amino acid composition of tryptic peptides from ovine growth hormone.

Amino acid	Peptide																			
	T_{1a}	T_{1b}	T_2	T_3	T_{2-3}	T_4	TA_{5a}	TA_{5b}	T_6	T_{7-8}	TC_8	T_9	T_{10}	T_{11-12}	T_{17}	T_{18}	TA_{18a}	T_{18-19}	T_{19-20}	T_{20}	T_{21}	T_{22}
1/2 Cys							N.D.									1.0	N.D.	1.0				
Asp	1.0		1.0		1.0		1.1		0.8	1.1		2.2		1.0	2.1	0.9	0.8	0.9	1.0	0.6		
Thr			1.1		1.0	0.8	1.2	1.9				2.1									1.8	
Ser	1.8						1.2	1.2		3.2	2.0	2.0			0.7	1.0	1.0	0.9				
Glu			2.3	1.9	4.4	2.1	1.8	1.2	3.3	3.1	1.9		1.1	2.1							1.2	
Pro	N.D.					1.0		2.1		0.7	0.8											
Gly	1.2					1.2		0.9		1.2	1.2	1.2		1.1		0.9	1.1	1.3				
Ala	1.5	0.9	2.9		2.6		1.1	1.0	1.0					1.0	1.1							
Val		1.0					0.7					1.9	0.8									1.0
Met	0.8													1.2								0.7
Ile						1.0	0.8	0.8		1.5	1.5			0.8								
Leu	2.4	1.1	1.7		1.8					8.3	5.3	1.3		3.5	1.9	2.0	2.0	2.1	1.2	1.0	1.0	
Tyr						1.0	0.6						0.8			0.9	0.9	1.0			0.9	
Phe	2.3		1.1	1.0	1.6		0.9	1.0		1.3	1.1	1.6				1.2		1.0				
Lys			1.0		1.1			0.7	0.9				1.0	0.9	0.9			0.8	1.6	1.0		1.0
His			1.8		2.0														1.1	1.0		
Arg		1.0		1.1	1.0	N.D.				1.8	N.D.	1.0		0.5		1.0		N.D.			N.D.	
Trp										*	*											

Uncorrected values obtained after 20 hr of hydrolysis, expressed as residues per mole of peptide. N.D., present but not determined.
* Detected by the Ehrlich reaction.

Table 2.
Amino acid composition of chymotrypic peptides from ovine growth hormone.

Amino acid	Peptide															
	C_1	C_2	C_3	C_7	C_8	C_{10-11}	C_{11-13}	C_{13}	C_{17}	C_{18}	C_{19}	C_{24}	C_{25}	C_{26a}	C_{26b}	C_{29-30}
1/2 Cys						0.7									0.9	
Asp			1.0				2.2	1.2	1.0	1.1	1.0	3.6	1.0			
Thr				1.2		2.1	1.9		1.0	1.0		1.1				
Ser	0.8	0.9				1.0	1.5	1.0	1.0	1.1		1.2			0.6	
Glu				1.0	2.1	1.1	5.4	3.5			2.9					
Pro	1.2				1.1	2.3	2.2									
Gly		0.9			1.1		1.3			0.8	1.1			1.0		
Ala	2.0		1.9			1.0	2.0	0.9				1.1				
Val			1.0						1.0	0.9						1.0
Met	1.0											0.7				1.3
Ile					0.8	0.8	0.9				0.8					
Leu	1.0	1.1	1.1				3.3	3.0	1.1		3.4	2.3		2.0		0.8
Tyr				0.9	0.8					0.7			0.9			
Phe	0.9	1.1				1.0			0.9						1.0	
Lys							1.5	1.0			1.5		1.1			
Arg				1.0	0.7					0.8		0.8				0.8

Uncorrected values obtained after 20 hr of hydrolysis, expressed as residues per mole of peotide.

pure condition peptide T_{7-8} (table 1) by the successive application of high voltage electrophoresis at pH 6.45 and chromatography with solvent 2 performed at right angles to the direction of the electrophoretic run.

2.3. *Chymotryptic peptides*

Were obtained and fractionated by ion-exchange chromatography, as described for bovine growth hormone [5]. Further purification of the isolated fractions was achieved by electrophoresis at pH 6.45 and two-dimensional chromatography with solvents 1 and 2.

2.4. *Peptic peptides*

The majority of these were obtained from a peptic digest of cyanogen bromide fragment 1 [1] submitted to high voltage electrophoresis at pH 6.45 and chromatography with solvent 2. Peptides P_{25a} and P_{25b} were obtained from a peptic digest of native hormone filtered through a Sephadex G-50 column as indicated by Zakin et al. [7]. The fractions containing the disulfide bridge not C-terminal, were reduced and carboxymethylated [8], filtered through a Sephadex G-25 column and submitted to partial acid hydrolysis [5]. By chromatography of the hydrolysate on a sulphonic acid resin [4] the peptides P_{25a} and P_{25b} were obtained in pure condition.

2.5. *Isolation of cystine-containing peptides*

A tryptic digest of the hormone was fractionated on Sephadex G-50 as indicated before for the isolation of tryptophan-containing peptides. The presence of half-cystine was detected by amino acid analysis after oxidation of the sample [5]. Those fractions whose amino acid compostion indicated that their half-cystine did not belong to the C-terminal disulfide bridge were pooled, reduced and blocked with ethylenimine [7]. The mixture was resolved on a Sephadex G-25 column (1.4 X 90 cm) equilibrated and eluted with 20% formic acid. The larger of the two peptides isolated was redigested with trypsin and the mixture purified by electrophoresis at pH 6.45 and paper chromatography with solvent 1.

2.6. *Nomenclature*

Peptides derived from tryptic, chymotryptic and peptic hydrolysates bear the prefix T, C or P, respectively, and are numbered in the order in which they occur in the sequence. Peptides derived from tryptic digestion of aminoethyl or citraconyl hormone bear the prefix TA or TC, respectively.

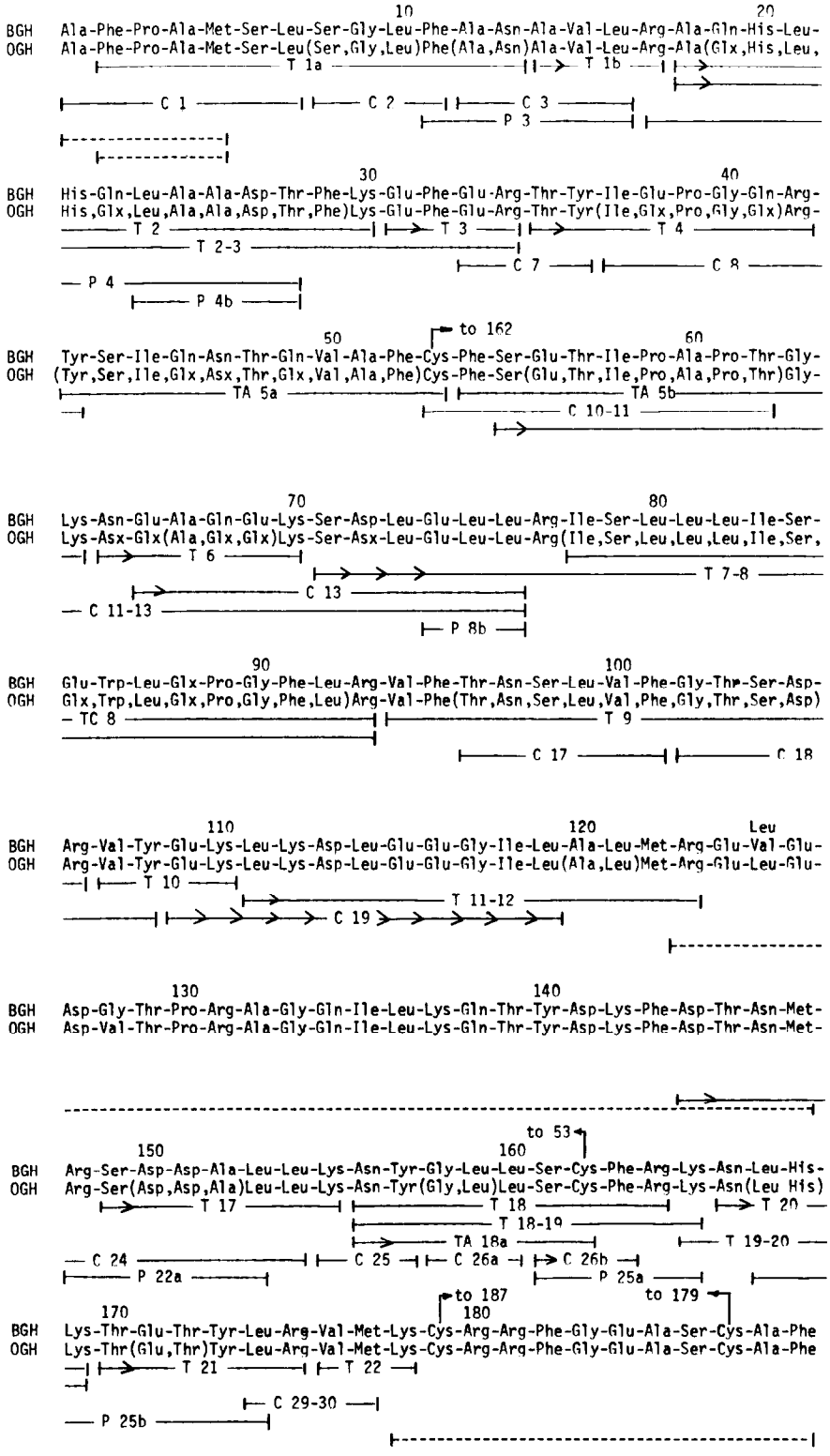

Fig. 1. Amino acid sequence of the ovine growth hormone molecule compared to that of bovine growth hormone [9]. The full line segments indicate tryptic (T, TA and TC), chymotryptic (C) and peptic (P) peptides. Dotted line segments correspond to cyanogen bromide fragments [1,4].→→Steps of Edman degradations.

Table 3
Amino acid composition of peptic peptides from ovine growth hormone.

Amino acid	Peptide						
	P_3	P_4	P_{4b}	P_{8b}	P_{22a}	P_{25a}	P_{25b}
1/2 Cys						1.0	
Asp	1.2	0.9	1.2		2.3		
Thr		1.2	1.1				1.9
Ser					1.3	0.8	
Glu		1.9		1.0			1.1
Ala	1.9	3.2	1.8		1.0		
Val	1.0						
Leu	1.2	1.9	0.8	2.1	1.2		2.1
Tyr							0.9
Phe	1.0					0.9	
Lys						1.0	1.0
His		2.1					1.1
Arg		0.8			0.8	1.1	

Uncorrected values obtained ater 20 hr of hydrolysis, expressed as residues per mole of peptide.

3. Results

3.1. *Tryptic peptides*

The amino acid compositions of the peptides studied are given in table 1. The assumed integral number of amino acid residues in each peptide is not shown but the following rule was applied: any fraction below 0.5 was considered 0 residue; any fraction equal or above 0.5 was considered 1 residue.

3.2. *Chymotryptic peptides*

The pattern of elution of these peptides from the ion-exchange column was almost identical to that obtained with the chymotryptic digest of bovine growth hormone [9]. The amino acid composition of all selected peptides is given in table 2.

3.3. *Peptic peptides*

Table 3 summarizes the analytical data corresponding to these peptides.

3.4. *Disulfide bridge not C-terminal*

The mixture resolved on the Sephadex G-25 column, as indicated in sect.2.5, contained the peptides originally joined by a disulfide bridge. These are the peptides indicated in table 1 as TA_{5a}, TA_{5b} and TA_{18a}.

4. Discussion

All the information collected in tables 1, 2 and 3 is organized in fig.1 to give a unique sequence of amino acids for the polypeptide chain of ovine growth hormone. Only the necessary peptides have been shown, although many more were studied but gave redundant information. The sequence shown incorporates also the structures, previously published, of the N- and C-terminal regions [1] and of a cyanogen bromide fragment [4]; in other sections of the chain besides these, the sequence has been established by the usual methods [5] or by considering the specificity of action of the proteolytic enzymes and the overlapping of peptides. The state of amidation of glutamyl and aspartyl residues could be decided, in some cases, from the electrophoretic mobility of the corresponding peptides.

Although the procedure applied in assembling the ovine growth hormone molecule by partial homology with bovine growth hormone forces a cautious interpretation of the results, the amount of absolute information incorporated gives assurance that the belief of near identity in the covalent structures of both hormones is solidly based.

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