ON THE PRIMARY STRUCTURE OF PITUITARY BOVINE GROWTH HORMONE

by

László Gráf and Choh Hao Li

Hormone Research Laboratory
University of California
San Francisco, California 94143

Received November 19,1973

SUMMARY: The primary structure of the tryptophan containing tryptic fragment obtained from bovine growth hormone has been determined. This sequence was found to be identical with that of the corresponding portion of sheep growth hormone. Together with other data, the corrected structure of bovine growth hormone is proposed herein.

Extensive studies (1,2,3,4) on the primary structure of bovine growth hormone (BGH) [1] have not yet led to an unambiguous result. From previous comparative structural studies on SGH (5) and BGH (3), only a single sequence difference - a Val/Gly interchange at sequence[2] position 130 - has been apparent (2,6). In contrast with this observation, the proposed sequences of SGH (5) and BGH (3,4) show many more differences than this single Val/Gly exchange. These are as follows:

	69	84	85	88	90	91	94	99
SGH:	Gln	Gln	Ser	Gly	Leu	Gln	Ser	Asp
BGH:	Glu	Ser	Gln	Gln	-	Gly	-	Asn

[1] Abbreviations: BGH, bovine pituitary growth hormone; SGH, sheep pituitary growth hormone; NBS, N-bromosuccinimide.

[2] All the residue numbers indicated in this paper correspond to the SGH structure (5).

It is remarkable that, with the exception of residues 69 and 99, all these differences are located in the tryptophan containing tryptic peptide, residues 78-95 of SGH. Therefore, we decided to prepare the corresponding fragment of BGH and re-examine its sequence. This paper reports the results of these studies. In addition, we propose the corrected amino acid sequence of the BGH molecule.

MATERIALS AND METHODS

BGH was prepared by the method previously described (7), except that this product was further purified by gel filtration on Sephadex G-100 (8). Trypsin, chymotrypsin, carboxypeptidase A, and aminopeptidase M were obtained from Calbiochem, Worthington, and Röhm-Haas, respectively. NBS was an Aldrich product.

Tryptic digestion of BGH was carried out in 0.01 M NH_4HCO_3 buffer of pH 9.0 with an enzyme to protein ratio of 1 : 50 (w/w) for four hours at 37° C. The tryptophan containing fragment was purified by gel filtration on Sephadex G-100 and partition chromatography on a column of Sephadex G-25 (9) with a solvent system of n-butanol-pyridine-0.1% aqueous acetic acid (40 : 24 : 96 by volume).

NBS cleavage of the tryptophanyl bond was carried out in 70% acetic acid with an approximate 5 molar excess of NBS over Trp content of peptide(s) for 30 minutes. To separate the cleavage products, the NBS reaction mixture was subjected to high voltage paper electrophoresis on Whatman 3MM paper in formic acid-acetic acid buffer of pH 2.1 for 60 minutes at 2000 V. Guide strips of the dried paper were developed with ninhydrin. The desired peptide bands were excised, eluted with 50% acetic acid, and evaporated.

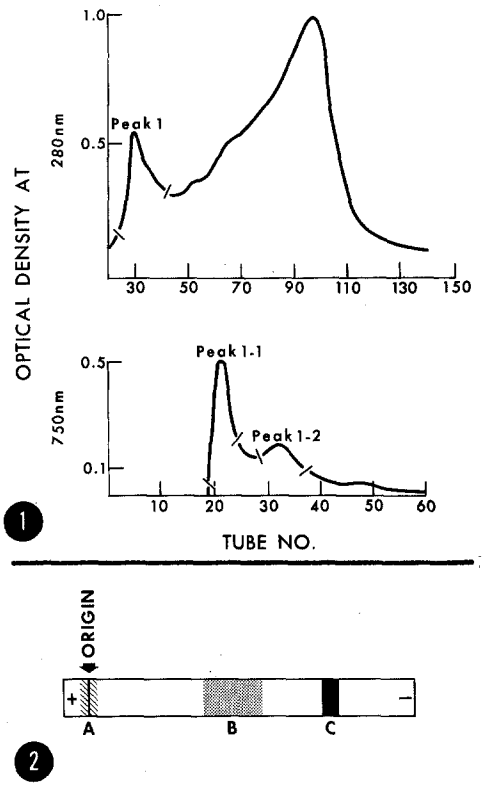

Figure 1: Upper: Chromatography of 400 mg of tryptic digest of BGH on a column (3.0 cm x 60 cm) of Sephadex G-100 in 0.01 M, pH 9.0, NH_4HCO_3 buffer. Tube volume was 5 ml. The yield of peak 1 was 29 mg.
Lower: Partition chromatography of 25 mg of peak 1 on a column (2.0 cm x 58 cm) of Sephadex G-25. Tube volume was 3 ml. The yield of peak 1-1 was 8 mg.

Figure 2: High voltage paper electrophoresis of NBS treated peak 1-1 at pH 2.1.

The homogeneity of the fragments obtained was examined by NH_2-terminal residue analysis (10). Amino acid analysis was carried out in an automatic amino acid analyzer (Model 120, Beckman Instruments). The amino acid sequence of the isolated peptides was determined by the dansyl-Edman method (10) as previously described (11). To distinguish the Glu/Gln residues in the sequence, a mixed enzymic digest of the peptide was analysed in the amino acid analyzer. This digestion was carried out with a mixture of

NH_2 - Ala - Phe - Pro - Ala - Met - Ser - Leu - Ser - Gly - Leu - Phe - Ala - Asn - Ala - Val - Leu - Arg - Ala - Gln - His -
1 10 20

Leu - His - Gln - Leu - Ala - Ala - Asp - Thr - Phe - Lys - Glu - Phe - Glu - Arg - Thr - Tyr - Ile - Pro - Glu - Gly -
30 40

Gln - Arg - Tyr - Ser - Ile - Gln - Asn - Thr - Gln - Val - Ala - Phe - Cys - Phe - Ser - Glu - Thr - Ile - Pro - Ala -
50 60

Pro - Thr - Gly - Lys - Asn - Glu - Ala - Gln - Gln - Lys - Ser - Asp - Leu - Glu - Leu - Leu - Arg - Ile - Ser - Leu -
70 80

Leu - Leu - Ile - Gln - Ser - Trp - Leu - Gly - Pro - Leu - Gln - Phe - Leu - Ser - Arg - Val - Phe - Thr - Asn - Ser -
90 100

Leu - Val - Phe - Gly - Thr - Ser - Asp - Arg - Val - Tyr - Glu - Lys - Leu - Lys - Asp - Leu - Glu - Glu - Gly - Ile -
110 120

Leu - Ala - Leu - Met - Arg - Glu - $\frac{\text{Val}}{\text{Leu}}$ - Glu - Asp - Gly - Thr - Pro - Arg - Ala - Gly - Gln - Ile - Leu - Lys - Gln -
130 140

Thr - Tyr - Asp - Lys - Phe - Asp - Thr - Asn - Met - Arg - Ser - Asp - Asp - Ala - Leu - Leu - Lys - Asn - Tyr - Gly -
150 160

Leu - Leu - Ser - Cys - Phe - Arg - Lys - Asp - Leu - His - Lys - Thr - Glu - Thr - Tyr - Leu - Arg - Val - Met - Lys -
170 180

Cys - Arg - Arg - Phe - Gly - Glu - Ala - Ser - Cys - Ala - Phe - OH
190

Figure 3: The amino acid sequence of bovine pituitary growth hormone.

chymotrypsin [1 : 50 (w/w) enzyme to peptide ratio], aminopeptidase M [1 : 20 (w/w) enzyme to peptide ratio], and carboxypeptidase A [1 : 20 (w/w) enzyme to peptide ratio] in 0.1 M $NaHCO_3$ for five hours at 37° C.

RESULTS

The tryptic digest of BGH was chromatographed on Sephadex G-100 as shown in Figure 1. Sequence studies on SGH (5) have shown that the Trp containing tryptic peptide is at least partly eluted with the void volume of the

Sephadex G-100 column. The presence of tryptophan could be detected in peak 1 (Figure 1) by spectrophotometric method and by Ehrlich reagent. The NH_2-terminal residue analysis of this fraction revealed Ile, Tyr, Phe, Ala, and Asx, indicating that it contains at least three tryptic fragments. In addition, a preliminary amino acid analysis of this peptide mixture strongly suggested that peak 1 (Figure 1) is composed of the Trp containing tryptic fragment (residues 78-95, NH_2-terminal Ile), the NH_2-terminal tryptic fragment (residues 1-17, NH_2-terminal Phe and Ala), and a disulfide containing tryptic peptide (residues 43-64 and 158-166, NH_2-terminal Tyr and Asn). The strong hydrophobic character of these fragments may explain the nature of their interactions, which result in the formation of a mixed aggregate eluted in peak 1 of Figure 1.

In an attempt to abolish these interactions, peak 1 (Figure 1) was subjected to partition chromatography as shown in the lower figure of Figure 1; peak 1-1 appeared with an R_f value of 0.95, which clearly demonstrates the strong hydrophobic character of the peptide(s) eluted. NH_2-terminal end residue and amino acid analyses showed that peak 1-1 contained the NH_2-terminal tryptic fragment, together with the Trp containing tryptic fragment, while the disulfide containing peptide of peak 1 was concentrated in peak 1-2 of Figure 1.

The material of peak 1-1 (Figure 1) remained at the origin when high voltage paper electrophoresis at pH 6.5 or 2.1, and paper chromatography in different solvent systems were used in an attempt to separate these two components. Since it was not possible to purify the Trp containing peptide in homogeneous form, peak 1-1 was treated directly with NBS and subsequently subjected to high voltage paper electrophoresis at pH 2.1. This procedure resulted in the appearance of a peptide fragment with high electrophoretic

mobility, designated as C in Figure 2. In addition to peptide C, both the diffuse band B and the material remaining at the origin (band A in Figure 2) were eluted and analysed for NH_2-terminal residues. The analysis revealed a single NH_2-terminal Leu for peptide C, and a single NH_2-terminal Ile for peptide A. On the other hand, band B was found to contain peptides with Leu, Phe, and Ala as NH_2-terminal residues. The amino acid composition of peptides A and C are shown in Table I. It is evident from Table I that peptide C represents the COOH-terminal portion of the Trp containing tryptic fragment (residues 87-95) released by NBS cleavage of the Trp-Leu bond (positions 86-87). Thus, the uncleaved but NBS-oxidized Trp containing tryptic peptide alone has remained at the origin during the high voltage paper electrophoresis at pH 2.1.

The results of the dansyl-Edman sequence determination of peptides[3] A and C were as follows:

peptide A: Ile - Ser - Leu - Leu - Leu - Ile - Glx - Ser - Trp - Leu -

peptide C: Leu - Gly - Pro - Leu - Glx - Phe - Leu - Ser - Arg

The NH_2-terminal sequence of peptide A is identical with the sequence of SGH between residues 78-87, and the sequence of peptide C is identical with residues 87-95 of the SGH sequence (5). From the amino acid analysis of a mixed enzymic hydrolysate of peptide A, it was apparent that both glutamic acids were amidated. Based on the above data, the amino acid sequence of the Trp containing tryptic peptide obtained from BGH is as follows:

Ile-Ser-Leu-Leu-Leu-Ile-Gln-Ser-Trp-Leu-Gly-Pro-Leu-Gln-Phe-Leu-Ser-Arg
78 80 85 90 95

[3] Instead of Trp, breakdown products were detected by the dansyl method.

Table I

Amino Acid Composition† of Peptides A and C from Figure 2

Amino Acid	Peptide A		Peptide C	
	Exp.	Theo. (78-95)**	Exp.	Theo. (87-95)**
Arg	1.2	1	0.9	1
Ser	2.8	3	0.7	1
Glx	2.3	2	1.0	1
Pro	0.8	1	0.9	1
Gly	1.3	1	1.0	1
Ile	1.7	2	-	-
Leu	6.0	6	2.8	3
Phe	1.2	1	1.0	1

† Compositions in molar ratio.

** Residue numbers in the SGH structure (5).

DISCUSSION

The unusual behavior of the Trp containing tryptic fragment in BGH during the preparation deserves further comment. The strong hydrophobic interaction of this peptide with others, in particular with the NH_2-terminal tryptic fragment, has made the sequence analysis difficult. This interaction could be destroyed by treating the peptide mixture obtained from peak 1-1 of Figure 1 with NBS.

From the above sequence data, it is evident that the structures of SGH and BGH between residues 78-95 are identical and correspond to the

sequence reported for SGH (5) rather than to the one proposed for BGH (2, 3).

Our structural investigations (12) on a small plasmic fragment of BGH originated from residues 65-70 of the structure gave evidence that the location of the amide groups within this peptide is the same as in the corresponding portion of the SGH structure (5) :

Asn - Glu - Ala - Gln - Gln - Lys
65 70

In addition, we recently reinvestigated (13) the sequence of a large tryptic fragment of BGH, residues 96-133, first isolated and examined by Yamasaki et al. (14, 15). The result of our sequence determination is identical to that reported by Yamasaki et al. (15), except that our sequence contains an extra leucine at position 121. Thus, the corrected amino acid sequence of BGH [4] is shown in Figure 3.

ACKNOWLEDGMENTS

The authors thank Jean Knorr and Daniel Gordon for their skilled technical assistance. This work was supported in part by the American Cancer Society.

REFERENCES

1. Santome, J. A., Dellacha, J. M., Paladini, A. C., Wolfenstein, C. E. M., Pena, C., Poskus, E., Daurat, S. T., Biscoglio, M. J., de Sese, Z. M. M., and Sanguesa, A. V. F. FEBS Letters 16, 198 (1971).
2. Fernandez, H. N., Pena, C., Poskus, E., Biscoglio, M. J., Paladini, A. C., Dellacha, J. M., and Santome, J. A. FEBS Letters 25, 265 (1972).

---

[4] During the preparation of this paper, we became aware of a similar revision for the BGH sequence reported briefly by Fellows (16), however, some of the Asn and Gln residues are not assigned in his proposed structure.

3. Santome, J. A., Dellacha, J. M., Paladini, A. C., Pena, C., Biscoglio, M. J., Daurat, S. T., Poskus, E., and Wolfenstein, C. E. M. Eur. J. Biochem. 37, 164 (1973).
4. Fellows, R. E., Rogol, A. D., and Mudge, A. Growth and Growth Hormone, edited by A. Pecile and E. E. Muller. Int. Congr. Ser. No. 244, p. 42. Excerpta Medica, Amsterdam (1972).
5. Li, C. H., Gordon, D., and Knorr, J. Arch. Biochem. Biophys. 156, 493 (1973).
6. Seavey, B. K., Singh, R. N. P., Lewis, U. J., and Geschwind, I. I. Biochem. Biophys. Res. Commun. 43, 189 (1971).
7. Li, C. H. J. Biol. Chem. 211, 555 (1954).
8. Bewley, T. A., and Li, C. H. Biochemistry 11, 927 (1972).
9. Yamashiro, D. Nature 201, 76 (1964).
10. Gray, W. R. Methods Enzymol. 11, 469 (1967); Woods, K. R., and Wang, K. T. Biochim. Biophys. Acta 133, 369 (1967).
11. Li, C. H., Dixon, J. S., Lo, T. B., Schmidt, K. D., and Pankov, Y. A. Arch. Biochem. Biophys. 14, 705 (1970).
12. Gráf, L., and Li, C. H. Arch. Biochem. Biophys., in press.
13. Gráf, L., and Li, C. H., manuscript in preparation.
14. Yamasaki, N., Kikutani, M., and Sonenberg, M. Biochemistry 9, 1107 (1970).
15. Yamasaki, N., Kangawa, K., Kobayashi, S., Kikutani, M., and Sonenberg, M. J. Biol. Chem. 247, 3874 (1972).
16. Fellows, R. E. Rec. Prog. Horm. Res. 29, 404 (1973).