Studies on the Common Active Site of Growth Hormone

REVISION OF THE AMINO ACID SEQUENCE OF AN ACTIVE FRAGMENT OF BOVINE GROWTH HORMONE

(Received for publication, August 12, 1974)

Nobuyuki Yamasaki and Juichiro Shimakaka
From the Laboratory of Biochemistry, Faculty of Agriculture, Ehime University, Matsuyama City, Japan
Martin Sonenberg
From the Sloan-Kettering Institute for Cancer Research, New York, New York

SUMMARY

A fragment, A-II, isolated from a component of a tryptic digest of bovine growth hormone has growth-promoting activity in rats and metabolic activity in humans similar to human growth hormone. The amino acid sequence of this peptide has been reinvestigated and revised. The 38-amino acid peptide was cleaved with cyanogen bromide, chymotrypsin, and trypsin. The amino acid sequences were then established by Edman degradation as well as with overlapping peptides. Homology in the sequence was good between this bovine growth hormone fragment and peptides occurring in ovine growth hormone, human growth hormone, and human chorionic somatomammotropin.

EXPERIMENTAL PROCEDURE

Materials

The active fragment A-II was prepared from a biologically active component of a tryptic digest of bGH as previously reported. From limited tryptic digests of bGH, a homogeneous component (3) has been isolated and dissociated to yield a large and a small fragment, A-I and A-II (4). The latter fragment has been characterized chemically and biologically (5-8). The amino acid sequence of A-II reported earlier (9) raised some question about the degree of homology among species and the sequence of bGH reported by others (10-13) in this active region of the hormone. For this reason the amino acid sequence has been re-examined.

Methods

Amino Acid Analysis—Amino acid analysis was performed by the method of Spackman et al. (17). The sample was hydrolyzed by constant boiling HCl in a sealed evacuated tube at 110° for 24 hours. On occasion, samples were also hydrolyzed for 48 and 72 hours. Amino acid analysis was carried out by use of a Hitachi amino acid autoanalyzer, type KLA-3B, and a Hitachi liquid chromatograph, model LS4.

Edman Deposition—Amino acid residues in the peptide were removed sequentially from the NH2 terminus by Edman’s method (18) with the use of the technique as reported by Blomhöck et al. (19). PTH derivatives were identified as previously reported (9). Amide groups were assigned after identification of the PTH by thin layer chromatography. Since deamination may occur during Edman degradation of long peptide chains, confirmations of assignments were obtained from the parent peptide as well as tryptic, chymotryptic, and cyanogen bromide fragments. No traces of PTH-Gln or PTH-Asn were noted when assignments of Gln and Asp, respectively, were made.

When a larger amount of A-II was submitted directly to Edman degradation, 4 μmol of A-II were divided into 2-μmol aliquots in two test tubes and the sequential degradation of A-II in test tubes was carried out simultaneously. After the 13th step of degradation, the remaining peptides were further purified. The peptides were combined and dissolved in a small amount of 50% acetic acid and applied on a column (1 X 40 cm) of Dowex 50 X 2 equilibrated with 0.2 M pyridine-acetate buffer, pH 5.6. With increasing pyridine concentration from 2.0 to 8.5 M, the effluents were subjected to alkaline hydrolysis and analyzed by ninhydrin reaction. In addition, the major fraction was collected and employed for further Edman degradation.

Determination of COOH-terminal Sequences—For some peptides, COOH-terminal sequences were determined by hydrazinolysis (20) and carboxypeptidase A digestion. The COOH-terminal amino acid obtained by hydrazinolysis was identified with an amino acid analyzer.
The peptide (0.3 to 0.5 μmol) dissolved in 0.2 M N-ethylmorpholine acetate buffer, pH 8.5, was allowed to react with DFP-treated carboxypeptidase A at 25°. At intervals, aliquots of the reaction mixture were taken and an equal volume of 10% acetic acid was added to terminate the reaction. The resulting solution was then evaporated and the released amino acids were determined on the amino acid analyzer.

Fragmentation and Purification of Peptides—The fragmentation of A-II by cyanogen bromide cleavage, chymotryptic and tryptic digestion, and the purification of the resulting peptides were performed as reported previously (9). The same designations for peptides described in the previous paper (9) were employed in these experiments.

RESULTS

Amino acid analysis (Table I) of A-II revealed approximately 0.7 mol of excess leucine compared to that previously reported (9). In addition, one of the cyanogen bromide fragments, CB-I, of A-II contained an additional leucine. Consequently, the integral number of amino acids in the active fragment, A-II, is estimated to be 38, and not 37 as previously reported (9).

When untreated A-II was submitted directly to Edman degradation, the remaining peptide was purified on a column of Dowex 50 X2 after 13 amino acids were removed sequentially from the NH2 terminus. The remaining peptide (Des-13 peptide) was tightly bound to the resin and was eluted by changing the buffer system to pH 5.6 and 8.5 M pyridine (Fig. 1). The NH2-terminal residue of the Des-13 peptide thus purified was found to be valine and further Edman degradation was performed with this peptide. In this way, the sequence of 26 amino acids from the NH2 terminus to leucine at position 26 in the sequence of A-II was determined (Table II). The amino acid sequence of the NH2 terminal part of A-II thus obtained agreed with the partial amino acid sequence determined with the cyanogen bromide fragment CB-I.

In Tables III and IV, the amino acid composition and the amino acid sequences of chymotryptic peptides are presented. The NH2-terminal residue of the chymotryptic peptide C-1-3 was found to be glutamic acid and neither methionine nor proline was found in this peptide. The COOH-terminal residue was found to be leucine by hydrazinolysis. On the basis of hydrazinolysis and carboxypeptidase digestion (Table V), the COOH-terminal sequence of this peptide was established as Gly-Ile-Leu. Edman degradation and COOH-terminal analysis established the sequence of C-I-3 as Glu-Lys-Leu-Lys-Asp-Leu-Glu-Gly-Ile-Leu. The NH2-terminal residue of the other chymotryptic peptide, C-1-4, was also found to be glutamic acid. A similar amino acid composition was recognized between C-I-3 and C-I-4 except for the additional amino acids alanine and leucine in C-I-4. Since the COOH-terminal residue of this peptide was found to be leucine by hydrazinolysis, alanine was placed between the two leucines. Thus, the COOH-terminal sequence of C-I-4 was established as Leu-Ala-Leu. This was confirmed by carboxy-

![Fig. 1. Purification of Des-13 peptide of A-II on Dowex 50-X2. Column size, 1 x 40 cm. Buffer systems for elution: (1) 100 ml of 0.2 M pyridine-acetic acid buffer, pH 3.2, were placed in a mixing chamber and 100 ml of 2.0 M pyridine-acetic acid buffer, at pH 5.0 were in the reservoir; (2) gradient was made by the combination of 50 ml of 2.0 M pyridine-acetic acid buffer, pH 5.0, in a mixing chamber and 50 ml of 8.5 M pyridine buffer at pH 5.6 in the reservoir; (3) the column was finally developed with 12 M pyridine buffer at pH 6.3. Chromatography was performed at 25° and one-ml fractions were collected.](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>Amino Acid Composition of A-II and its cyanogen bromide fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid composition of A-II and its cyanogen bromide fragments</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Amino acid</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Homoserine lactone</td>
</tr>
<tr>
<td>Total residues</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Values are expressed as the average of four experiments. Peptides were hydrolyzed for 24, 48, and 72 hours and appropriate corrections have been made for amino acids partially destroyed during hydrolysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanogen bromide fragments of A-II.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence of 20 amino acids from NH2 terminus in A-II and yield of PTH at each step of Edman degradation</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Yield of PTH was calculated spectrophotometrically by using the extinction coefficient of each PTH derivative. At the position marked by ↓, the remaining peptide was purified. See details in text.</td>
</tr>
</tbody>
</table>

| Amino Acid | Val-Ph-Thr-Asn-Asp-Glu-Val-Phe-Glu-Thr |
|---------------------------------------------------------------|
| Yield (%) | 100 89 90 74 63 67 68 61 56 39 |
|---------------------------------------------------------------|
| Amino Acid | Ser-Asp-Arg-Glu-Tyr-Glu-Lys-Leu-Lys-Asp |
|---------------------------------------------------------------|
| Yield (%) | 28 27 26 11 10 9 9 7 10 7 |
|---------------------------------------------------------------|
| Amino Acid | Leu-Glu-Glu-Glu-Ile-Leu |
|---------------------------------------------------------------|
| Yield (%) | 7 6 7 6 5 |
Amino acid composition of chymotryptic peptides in Fraction C-I

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>C-I-1</th>
<th>C-I-2</th>
<th>C-I-3</th>
<th>C-I-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-I-1</td>
<td>Met-Arg-Glu-Leu-Glu-Asp-Gly-Thr-Pro-Arg</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.82 (2)</td>
<td>2.14 (2)</td>
</tr>
<tr>
<td>C-I-2</td>
<td>Ala-Leu-Met-Arg-Glu-Leu-Glu-Asp-Gly-Thr-Pro-Arg</td>
<td>1.70 (2)</td>
<td>1.92 (2)</td>
<td>0 (0)</td>
<td>1.00 (1)</td>
</tr>
<tr>
<td>C-I-3</td>
<td>Glu-Lys-Leu-Lys-Asp-Glu-Gly-Ile-Leu</td>
<td>1.00 (1)</td>
<td>1.00 (1)</td>
<td>1.00 (1)</td>
<td>1.00 (1)</td>
</tr>
<tr>
<td>C-I-4</td>
<td>Glu-Lys-Leu-Lys-Asp-Glu-Gly-Ile-Leu-Ala-Leu</td>
<td>0.72 (1)</td>
<td>0.85 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Completed in the previous paper (9)

Corrected in the present experiment.

**Table IV**

Amino acid sequence of chymotryptic peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-I-1</td>
<td>Met-Arg-Glu-Leu-Glu-Asp-Gly-Thr-Pro-Arg</td>
</tr>
<tr>
<td>C-I-2</td>
<td>Ala-Leu-Met-Arg-Glu-Leu-Glu-Asp-Gly-Thr-Pro-Arg (*)</td>
</tr>
<tr>
<td>C-I-3</td>
<td>Glu-Lys-Leu-Lys-Asp-Glu-Gly-Ile-Leu (**)</td>
</tr>
<tr>
<td>C-I-4</td>
<td>Glu-Lys-Leu-Lys-Asp-Glu-Gly-Ile-Leu-Ala-Leu (**)</td>
</tr>
<tr>
<td>C-II-1</td>
<td>Met-Arg-Glu-Leu-Glu-Asp-Gly-Thr-Pro-Arg</td>
</tr>
<tr>
<td>C-II-2</td>
<td>Gly-Thr-Arg-Val-Pro-Arg</td>
</tr>
<tr>
<td>C-II-3a</td>
<td>Thr-Asn-Val-Leu-Phe</td>
</tr>
<tr>
<td>C-II-3b</td>
<td>Val-Phe-Gly-Val-Asp-Arg-Val-Tyr</td>
</tr>
<tr>
<td>C-II-4</td>
<td>Val-Phe</td>
</tr>
<tr>
<td>C-II-5a</td>
<td>Val-Phe-Val-Asp-Arg-Val-Tyr</td>
</tr>
<tr>
<td>L-II-5b</td>
<td>Val-Phe-Val-Asp-Arg-Val-Tyr</td>
</tr>
</tbody>
</table>

* Completed in the previous paper (9)

** Corrected in the present experiment.

peptidase A digestion (Table V). Edman degradation and analysis of the COOH-terminal sequence revealed the sequence in C-I-4 as Glu-Lys-Leu-Lys-Asp-Leu-Glu-Gly-Ile-Leu-Ala-Leu. This sequence supported the amino acid sequence from glutamic acid at 16 to leucine at 26 which was previously determined by repeated Edman degradation of untreated A-II. The NH₂-terminal portion of the peptide C-I-2 overlapped the COOH-terminal sequence of C-I-4 (Table IV). The established amino acid sequence of C-I-1 and C-I-2 supported the COOH-terminal sequence of A-II which was determined previously (9) with the cyanogen bromide fragment CB II. The other chymotryptic peptides in the group of C-II were all consistent with the portion in the sequence of A-II already established with Edman degradation of untreated A-II and with chymotryptic peptides in the C-I fraction.

To establish further the structure of A-II as determined above, tryptic peptides were employed. The amino acid analyses of the tryptic peptides were reinvestigated (Table VI) and the amino acid sequences of these peptides were determined by Edman degradation. As shown in Table VI, a tryptic peptide designated as T-I-1 was found to extend from glutamic acid to the COOH-terminal residue of A-II. Another peptide, T-I-2, has aspartic acid as an NH₂-terminal residue and this peptide was found to correspond to the peptide extending from aspartic acid at position 20 to arginine at position 30 in the sequence of A-II. This had already been established by alignment of the chymotryptic peptides as C-I-3, C-I-2, C-I-4, and C-I-1 and by successive Edman degradation of untreated A-II. The amino acid composition of the larger peptide, T-I-1, showed good agreement with that of the peptide between the NH₂ terminus and lysine at position 19. Another peptide, T-I-1b, was also found to be a peptide from the NH₂ terminus to arginine at 13 in the sequence of A-II. The amino acid sequence of T-I-1 supported the sequence around a tyrosine residue at position 15. The established amino acid sequence of a tryptic peptide designated as T-I-2
was consistent with the peptide from leucine at position 18 to 
arginine located at the carboxyl end of methionine.

All results described above established the revised amino acid 
sequence of an active fragment of bGH (Fig. 1). The molecular 
weight of this fragment was calculated to be 4343 from this 
sequence and this value is close to the value 4500 estimated by gel filtration (5).

**DISCUSSION**

As has been reported (1-4, 6, 7, 21), significant amounts of 
growth-promoting and metabolic activity are retained when 
growth hormone is digested with trypsin. These observations 
reveal the finding that such tryptic digests of bGH have 
growth-promoting and metabolic activity are retained when 
arginine located at the carboxyl end of methionine.

The homogeneity of an isolated component of a tryptic digest of bGH with metabolic 
activity in humans similar to hGH has been established by gel 
filtration, sedimentation equilibrium, and disc electrophoresis 
(3). This biologically active component could be separated into 
the purified remaining peptide, A-I and A-II, by gel filtration in 50% acetic acid 
(4). On the basis of the amino acid composition and the terminal amino acid residues of these fragments, we suggested that the 
biologically active component of tryptic digests of bGH was 
composed of two peptides which were obtained by tryptic hy-
drolysis at an arginyl valine and an arginyl-serine peptide bond 
in a segment of the polypeptide chain within the large disulfide 
loop of the bGH molecule (4) We also demonstrated that the 
smaller fragment, A-II, had growth-promoting activity in rats 
(4), metabolic activity in hypopituitary humans (7, 8), and in 
vitro activity with human erythrocyte membranes (22) similar to 
hGH.

In the previous paper (9) we showed the similarity in the 
amino acid sequence between A-II and the peptide occurring 
from valine 96 to arginine at 133 in the amino acid sequence of 
bGH (23).

In the present experiment, a larger amount of A-II peptide was 
employed for direct Edman degradation and after the 13th step 
of degradation, the remaining peptide was purified on Dowex 
50-X2. The further sequential removal of amino acid residues in 
the purified remaining peptide gave the amino acid sequence from 
the NH2 terminus to leucine at position 26 in the sequence of 
A-II. The alignment of chymotryptic peptides as C-I-3, C-I-2, 
C-I-4, and C-I-1 gave the sequence from glutamic acid at 16 to 
the COOH terminus arginine of A-II as determined above. 
Chymotryptic peptides C-I-1 and C-I-2 also confirmed the 
COOH-terminal sequence previously determined with the nona- 
peptide which was obtained by cyanogen bromide cleavage of 
A-II (9). The completely sequenced tryptic and other chymo-
tryptic peptides gave the overlapping peptides and they sup-
ported the amino acid sequence of A-II.

Recently, Santoné et al. (10) presented the primary structure 
of bGH, in which A-II was found to correspond with the peptide 
96-133 of their sequence except for some discrepancy. Our 
present study revises the amino acid sequence of A-II (Fig. 2) 
and confirms the previous sequences (10-13). The amino acid 
sequence of A-II is also homologous with that of the peptide 
96-133 of ovine growth hormone (24) and 95-133 of hGH (25, 26) 
and human chorion somatomammotropin (26-28). Some 
replacements of amino acids are recognized in the corresponding 
positions (Fig. 3). However, most of them are consistent with 
amino acid pairs with highly favored codon substitutions (29).

Recently, an N'-acetyl peptide with 42 amino acids cor-
responding to the peptide 95-136 in the sequence of hGH was 
synthesized and found to have growth-promoting activity in rats 
(30). Chillemi and Pecile (31) have also presented evidence that 
synthetic peptides 81-121 and 122-153 in the hGH sequence have 
growth hormone activity. It is of further interest that the syn-
thetic nonapeptide corresponding to the COOH-terminal se-
quence of A-II has also been found to possess growth hormone 
activity as revealed by the tibia width test in rats (32).

**Fig. 2.** The revised amino acid sequence of an active fragment 
of bovine growth hormone. *Numbers below the lines* indicate the 
position of the amino acid residue from the NH2 terminus. *C*, 
points of chymotryptic attack; *T*, points of trypptic attack; *CB*, 
points of attack by cyanogen bromide.

A-II 

BGH Val-Phe-Thr-Asn-Ser-Leu-Val-Phe-Gly-Thr-Ser-Asp-Arg-Val-Tyr-

OGH Val-Phe-Thr-Asp-Ser-Leu-Val-Phe-Gly-Thr-Ser-Asp-Arg-Val-Tyr-

HGH Val-Phe-Ala-Asn-Ser-Leu-Val-Tyr-Gly-Ala-Ser-Asn-Ser-Asp-Arg-Leu-

HCS Met-Phe-Ala-Asn-Ser-Leu-Val-Tyr-Asp-Thr-Ser-Asp-Arg-Asp-Tyr-His-Leu-

A-II 

BGH Asp-Leu-Glu-Gly-Ile-Leu-Ala-Leu-Met-Arg-Glu-Leu-Glu-Gly-Thr-Pro-Arg 

OGH Asp-Leu-Glu-Gly-Ile-Leu-Ala-Leu-Met-Arg-Glu-Leu-Glu-Gly-Thr-Pro-Arg 

HGH Asp-Leu-Glu-Gly-Ile-Leu-Thr-Leu-Met-Gly-Arg-Leu-Glu-Gly-Gly-Ser-Pro-Arg 

HCS Asp-Leu-Glu-Gly-Ile-Leu-Thr-Leu-Met-Gly-Arg-Leu-Glu-Gly-Gly-Ser-Pro-Arg 

**Fig. 3.** Comparison of amino acid sequence of peptides from tryptic di-
gest of bovine growth hormone (A-II (BGH)), ovine growth hormone 
(OGH), human growth hormone (HGH), and human chorion sona-
tomammotropin (HCS). *Numbers below the lines* indicate the position 
of the amino acid residue in the sequence of each protein molecule.
Because of the similarities of metabolic effects elicited by various pituitary growth hormones in humans and lower species, it has been considered that there may be a common active portion in all growth hormone molecules. The results described herein all support our previously reported postulations (3, 4) that the structure of the "active site" of bGH may be common to growth hormones of several species and this site may be confined to a relatively small portion of the large polypeptide chain within the large disulfide loop of the parent molecule.

REFERENCES

5. YAMASAKI, N. & KANGAWA, K. (1973) Agric. Biol. Chem. 37, 2631-2637
27. DAYhoff, M. O., ECK, R. V. (1969) in Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Silver Spring, Maryland
31. DAYhoff, M. O., ECK, R. V. (1969) in Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Silver Spring, Maryland
Additions and Corrections

Requirements for Parathyroid Hormone Action in Renal Membranes with the Use of Inhibiting Analogues.


On Page 3199, the name of the first author should be David Goltzman

Page 7979, Step 6, third sentence should read, instead of “...from the cathodal end...”:

Aldolase was eluted from the anodal end....

Vol. 250 (1975) 3199-3203

In Goltzmann, David, André Peytreman, Edward Callahan, Geoffrey W. Tregear, and John T. Potts, Jr. Analysis of the

Vol. 250 (1975) 2510-2514

In Yamasaki, Nobuyuki, Juichiro Shimanaka, and Martin Sonenberg. Studies on the Common Active Site of Growth Hormone. Revision of the Amino Acid Sequence of an Active Fragment of Bovine Growth Hormone.

Page 2512, Table III, Line 2, arginine residues under Column 4, peptide C-1-4, should read 0 (0)

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
Studies on the common active site of growth hormone. Revision of the amino acid sequence of an active fragment of bovine growth hormone.

N Yamasaki, J Shimanaka and M Sonenberg


Access the most updated version of this article at http://www.jbc.org/content/250/7/2510

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/7/2510.full.html#ref-list-1