The C-Terminal Amino Acid Sequence of Growth Hormones
from Human, Monkey, Whale, and Sheep Pituitary Glands*

CHOH HAO LÜ, ALAN J. PARCELLS†, AND HAROLD PAPKOFF

From the Hormone Research Laboratory and the Department of Biochemistry,
University of California, Berkeley, California

(Received for publication, July 28, 1958)

In another communication we have reported partial N-terminal amino acid sequences for growth hormones (somatotropins) of several species (1); these studies have indicated that whale, monkey, and human growth hormones are single-chain polypeptides at their N-terminus. To seek further evidence that these proteins are made up of single polypeptide chains, as well as to make additional comparisons with beef and sheep growth hormones, investigations at the C-terminus of these proteins have been carried out by enzymic and chemical means. Results of these investigations are reported herein.

EXPERIMENTAL

Sheep, whale (humpback), monkey (rhesus, Macaca mulatta), and human growth hormones were isolated from pituitary glands by previously published procedures (2–5). No evidence of inhomogeneity was observed when these protein preparations were submitted to studies of purity by various physicochemical techniques (3–6). The growth-promoting activity of the hormone was estimated by the tibia test (7) in hypophysectomized rats.

The crystalline carboxypeptidase was a commercial preparation from Worthington Biochemical Corporation. Enzymic digestion was carried out as follows. Growth hormone preparations were incubated with carboxypeptidase in an enzyme to substrate molar ratio of 1:25; the volume was 2 ml. in 1 per cent NaHCO₃ with the concentration of substrate 0.4 μmole. The enzyme was pretreated with diisopropyl fluorophosphate to ensure the complete absence of endopeptidase (8). Digestion was carried out at 25°C. Aliquots were taken at intervals with a 500 μl. micropipette and transferred to a 10 × 75-mm. culture tube containing 800 μl. of 2 per cent fluorodinitrobenzene in ethanol. The reaction was allowed to proceed for 2 hours with constant shaking on a mechanical shaker. The reaction mixture was then diluted to 6 ml. and excess fluorodinitrobenzene was extracted with three 5-ml. portions of ether. The aqueous solution was then acidified with several drops of concentrated HCl and extraction with 5-ml. portions of ether was again performed until there was no yellow color in the extracts. The pooled ether extracts were evaporated to dryness and submitted to two-dimensional paper chromatography according to the procedure of Levy (9).

The procedure of Niu and Fraenkel-Conrat (10) for the hydrazinolysis (11) of proteins has been employed exactly as these authors have described it, with the exception that an additional extraction of the alkaline reaction mixture with ethyl acetate was performed to ensure removal of 2,4-dinitrophenylhydrazides.

RESULTS AND DISCUSSION

It can be seen in Table I that the growth hormones under investigation all seem to possess C-terminal phenylalanine to the extent of 1 mole per mole of protein. A study of the rate of liberation of the various amino acids by carboxypeptidase yields some information about the C-terminal amino acid sequences of somatotropin obtained from the various species. With the ovine hormone, the amino acid that is cleaved the most rapidly by the enzyme after phenylalanine is leucine, and then alanine and serine are released in that order (Fig. 1). Hence, the C-terminal amino acid sequence of . . . Ala. Leu. Phe is proposed for ovine growth hormone. When whale growth hormone is subjected to digestion, a different sequence is indicated, since the alanine was found to be liberated at almost the same rate as the phenylalanine (Fig. 2). With a different sample of hormone from the same species, alanine and leucine were found to be liberated in almost equal amounts, a finding duplicated with a performic acid-oxidized preparation. The data, however, do indicate that alanine is being liberated by carboxypeptidase at a faster rate than leucine. Hence the sequence . . . Leu. Ala. Phe is proposed for the C-terminus of whale growth hormone. Similarly, from the data in Figs. 3 and 4 the C-terminal amino acid sequences . . . (Ala, Gly). Phe and . . . Leu. Phe may be postulated for monkey and human growth hormones, respectively.

When hydrazinolysis (10, 11) was used to verify the findings derived from the experiments with carboxypeptidase to the effect that phenylalanine is the sole C-terminal residue in growth

* Taken from the thesis submitted by Alan J. Parcells in partial fulfillment of requirements for the degree of Doctor of Philosophy, University of California, June, 1958.

† Present address, Laboratory for the Study of Hereditary and Metabolic Disorders, University of Utah College of Medicine, Salt Lake City, Utah.

1 The whale hormone was oxidized by performic acid according to the procedure previously described (12). No cysteic acid was liberated from the oxidized hormone by the action of carboxypeptidase. If cysteic acid has become the C-terminal residue after oxidation with performic acid, it will be cleaved by carboxypeptidase, as has been demonstrated by experiments with performic acid-oxidized prolactin (13).

2 A similar sequence has been observed in the C-terminus of bovine growth hormone (14).
TABLE I
Quantitative estimation of amino acids liberated by action of carboxypeptidase on somatotropin

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample No.</th>
<th>Amount used</th>
<th>Time of digestion</th>
<th>Amino acid liberated</th>
<th>Liberation of phenylalanine†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg. hrs. μmol</td>
<td>μmol</td>
<td>μmol</td>
<td>μmol</td>
</tr>
<tr>
<td>Ovine</td>
<td>1</td>
<td>4.6 6</td>
<td>0.097 0.023 0.032</td>
<td>0</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.6 6</td>
<td>0.093 0.023 0.044</td>
<td>0</td>
<td>0.95</td>
</tr>
<tr>
<td>Cetacean</td>
<td>1</td>
<td>4.2 5</td>
<td>0.139 0.072 0.060</td>
<td>0</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.1 6</td>
<td>0.115 0.102 0.035</td>
<td>0</td>
<td>1.10</td>
</tr>
<tr>
<td>Simian</td>
<td>1</td>
<td>2.5 4</td>
<td>0.000 0.025 0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.0 8</td>
<td>0.075 0.030 0</td>
<td>0</td>
<td>0.68</td>
</tr>
</tbody>
</table>

† Enzyme-substrate molar ratio of 1:25; incubated at pH 8.3 and 25°C.

Assumed molecular weights of various somatotropins were as follows (3-6): ovine, 47,000; cetacean, 40,000; simian, 26,000; human 27,000.

---

hormone preparations, no amino acids resistant to the action of the enzyme (e.g., lysine, arginine, or proline) were detected. As might be expected, phenylalanine was found to be the predominant amino acid released by hydrazinolysis, although traces of other amino acids were observed. The value calculated for the recovery of phenylalanine was subjected to hydrazinolysis without any protein. When the losses from destruction in the presence and absence of protein were multiplied together, a recovery factor of 2.92 was obtained. Accordingly, the corrected yield of the C-terminal phenylalanine residue in the ovine hormone is 0.96 mole per mole of protein, and in the human growth hormone, 0.8 mole.† These findings, in conjunction with the results of the carboxypeptidase experiments, clearly show that there is only 1 C-terminal phenylalanine residue in ovine and human growth hormones.

† It may be recalled that a value of 0.9 mole for C-terminal phenylalanine was obtained by earlier hydrazinolysis of beef growth hormone (15).

---

FIG. 1. Rate of release of amino acids by the action of carboxypeptidase on ovine somatotropin. Enzyme-substrate, 1:25; pH 8.4, at 25°C.

FIG. 2. Rate of release of amino acids by the action of carboxypeptidase on whale somatotropin. Enzyme-substrate, 1:25; pH 8.4, at 25°C.

FIG. 3. Rate of release of amino acids by the action of carboxypeptidase on monkey somatotropin. Enzyme-substrate, 1:25; pH 8.4, at 25°C.

FIG. 4. Rate of release of amino acids by the action of carboxypeptidase on human somatotropin. Enzyme-substrate, 1:25; pH 8.4, at 25°C.

TABLE II
Growth-promoting activity of carboxypeptidase-treated somatotropin

<table>
<thead>
<tr>
<th>Species</th>
<th>Time of digestion</th>
<th>No. of rats</th>
<th>Width of uncalcified tibia cartilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovine</td>
<td>hrs.</td>
<td>6</td>
<td>218 ± 3†</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6</td>
<td>214 ± 3†</td>
</tr>
<tr>
<td>Cetacean</td>
<td>6</td>
<td>10</td>
<td>237 ± 12†</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>242 ± 6†</td>
</tr>
<tr>
<td>Simian</td>
<td>6</td>
<td>6</td>
<td>236 ± 3†</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10</td>
<td>245 ± 3†</td>
</tr>
</tbody>
</table>

† Animals given a total dose of 0.04 mg. in 4 days.
‡ Mean ± standard error.
When somatotropins from which 1 residue of phenylalanine had been removed by the action of carboxypeptidase were assayed for growth-promoting activity by the tibia test (7), it was found that C-terminal phenylalanine is not essential for the biological function of growth hormones (Table II). These observations are consistent with earlier studies with the bovine hormone (14).

**SUMMARY**

By means of a combination of two methods, enzymic digestion with carboxypeptidase, and hydrazinolysis, it has been possible to deduce the following C-terminal amino acid sequences for growth hormones from several species: sheep, ... Ala.Leu.Phe; whale (humpback), ... Leu.Alp.Phe; monkey (rhesus, *Macaca mulatta*), ... (Ala,Gly).Phe; human, ... Leu.Phe. Moreover, it can be shown that the C-terminal phenylalanine residues of these growth hormones are not essential for their biological activity in terms of assay by the tibia test.

**Acknowledgments**—The authors wish to acknowledge the generosity of the American Cancer Society, and the Albert and Mary Lasker Foundation for research grants.

**REFERENCES**

The C-Terminal Amino Acid Sequence of Growth Hormones from Human, Monkey, Whale, and Sheep Pituitary Glands
Choh Hao Li, Alan J. Parcells and Harold Papkoff