hormonal proteins and peptides

EDITED BY CHOH HAO LI

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HORMONAL PROTEINS AND PEPTIDES

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Preface

The importance of the hypophysis in the control of growth in man has been known for a long time. In 1886, P. Marie reported a patient with somatic overgrowth (acromegaly), and associated this condition with an abnormality of the pituitary gland. Thirty years later J. Erdheim elucidated the role of the hypophysis in pituitary dwarfs. Experimental demonstrations for the relationship of this gland to growth began in 1910 with the report of S. J. Crowe, H. Cushing, and J. Homans on the hypophysectomy of dogs. These investigators concluded that total removal of the pituitary was fatal, but in the few dogs that survived for several months the body growth was retarded. In 1921, H. M. Evans and J. A. Long observed a resumption of growth in normal adult rats when treated with pituitary extract. Later, P. E. Smith in 1930 implanted pituitary tissue under the skin of hypophysectomized rats and discovered that the animals resumed their growth and regained their lost functions. These early experiments provided evidence for the existence of growth-promoting substances in the pituitary gland. The isolation of growth hormone in highly purified form from bovine pituitary glands (BGH) was achieved in 1944. Since BGH preparations substantially free from other pituitary hormones have become available, it has been possible to define the biological effects of growth hormone in experimental animals. When a pituitary dwarf was treated with BGH in 1950, no effects on calcium, phosphorus, or nitrogen were observed. The reason BGH was not effective in man was understood when highly purified growth hormone from human pituitary glands (HGH) was first isolated in 1956 and shown to be chemically distinct from the bovine hormone.

This volume presents five chapters on various aspects of growth hormone. In the opening article, the chemistry of the HGH molecule is discussed with special regard to the relationship between structure and biological activity. In the second review, Hayashida gives a comprehensive account of immunological and biological studies with antisera to growth hormone from various species. The subject has rapidly expanded since the first report on the production of antibody to HGH in the rabbit in 1958. This review includes immunoassay of growth hormone in blood

plasma and immunochemical comparisons of growth hormones from mammalian and submammalian vertebrate species. In the third paper, Escamilla summarizes his experiences on the clinical studies of HGH in children with growth problems. It is now established that only primate growth hormones are active in man and that hypopituitary dwarfs can be successfully treated with HGH. In addition, it has recently been demonstrated that HGH exhibits beneficial effects on patients with bleeding ulcers and other metabolic diseases.

In the fourth chapter, Lawrence and his co-workers review the clinical state of acromegaly and recent medical therapy to lower HGH levels in patients. Since 1958 these investigators have used heavy particles to treat more than 275 patients with pituitary tumors. This is one of the landmarks in nuclear medicine. In the concluding article, Bennett gives an account of the development of pituitary research with a biographical sketch of Evans, a twentieth-century pioneer in experimental biology. Bennett knew Evans for nearly forty years, and is himself an active investigator of pituitary hormones related to carbohydrate metabolism.

I wish to expess my appreciation to the staff of Academic Press for their cooperation in the preparation of this volume and in the important task of preparing the Subject Index.

Choh Hao Li

Contents

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The Chemistry of Human Pituitary Growth Hormone: 1967–1973

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I. Introduction

In 1967 (1), I summarized the chemistry of HGH* after its first isolation and partial characterization in 1956 (2,3). This paper reviews the recent data on the same subject which have been published during the

* Abbreviations: HGH, human pituitary growth hormone; SGH, sheep pituitary growth hormone; BGH, bovine pituitary growth hormone; RCAM-, reduced-carba-

period 1967–1973. It should be noted that important progress has been made, especially on the relationship between chemical structure and biological activity. In addition, the synthesis of HGH-like activity has been achieved.

Table I presents the known physicochemical properties of HGH. It is a globular protein† with a molecular weight of 22,000, isoelectric point

midomethylated derivative; RCOM-, reduced-carboxymethylated derivative; NPS-, *o*-nitrophenyl sulfenyl derivative; HNB-, 2-hydroxy-5 nitrobenzyl derivative; NPS-Cl, *o*-nitrophenylsulfenyl chloride, HNB-Br, 2-hydroxy-5-nitrobenzyl bromide; ORD, optical rotatory dispersion; CD, circular dichroism; Bpoc, 2-(biphenyl)-isopropyloxycarbonyl; Boc, *t*-butyloxycarbonyl.

† In disc electrophoresis experiments (50 γ, 4 mA/ml, 60 minutes), HGH migrates as a single component in a buffer of pH 4.5 as shown in the figure. However, it gives one major component and a minor fast moving component at pH 8.3; the minor component is identified as the deamidated HGH.

Disc electrophoresis of HGH in 7.5% polyacrylamide gel.

Table I—Physicochemical Properties of HGH

Molecular weight	
Sedimentation equilibrium	21,500
Osmotic pressure	22,100
Primary structure	22,125
Isoelectric point, pH	4.9
Sedimentation coefficient, $s_{20,w}$	2.179
$[\alpha]^{25}$ (0.1 *M* acetic acid)	−38.7
$E^{0.1\%}_{1\text{ cm, }277\text{ nm}}$	0.931
$[\theta]$ 221 nm, pH 8.2	−19,700
α-Helical content, %	55
Partition coefficient (2-butanol-0.1% DCA)	1.44

at pH 4.9, and α-helical content of 55%. In addition to the well-established clinical usefulness of HGH for hypopituitary children (4), it has been demonstrated that the hormone exhibits beneficial effects on patients with bleeding ulcers (5) and has potential for aiding muscular dystrophy (6). Friedman *et al.* (7) reported the hypocholesterolemic effect of HGH in coronary prone hypercholesterolemic subjects. The serum cholesterol content of each of the four subjects fell following the 7-day administration of HGH, only to rise to their preinjection levels during the 3 weeks following cessation of the HGH injections. The fall in serum cholesterol during HGH administration was accompanied by a significant rise in the serum triglyceride level of each subject. The pre-β-lipoprotein fraction of the lipoprotein spectrum also rose significantly. However, the fasting serum free fatty acid levels of these subjects showed no change.

Some of the *in vivo* effects of HGH are mediated via somatomedin (8), particularly its action on bony tissues. It is unlikely that every biological

Table II—*In Vitro* Effects of HGH

Effect	Tissue
An increased rate of nuclear multiplication	Human liver cells (9)
Stimulation of amino acid incorporation into protein	Rat diaphragms (10)
Inhibition of glucose consumption	Human erythrocytes (11)
Increase glucose consumption and lactic acid accumulation	Human dermal fibroblasts (11a)
Fast lipolytic action	Rabbit fat cells (12)
Stimulation of DNA, RNA, and protein synthesis	Human leukemic cells (13)
Increase of blastic transformation	Human lymphocytes (14)

Table III—Stimulation of Lipolysis in Rat and Rabbit Fat Cells by HGH and ACTH

Hormone	Concentration (10^{-6} *M*)	Glycerol production[a]	
		Rat[b]	Rabbit[c]
None	0	0.9 ± 0.04	1.6 ± 0.2
HGH	1.13	0.9 ± 0.10	6.5 ± 0.3
	4.50	1.3 ± 0.10	24.1 ± 0.6
ACTH	0.03	24.3 ± 0.2	22.9 ± 0.5

[a] mean ± S.E.
[b] μmoles glycerol/gm cells/2 hour.
[c] μmoles glycerol/gm cells/1 hour.

activity of the hormone depends upon the production of somatomedin. Table II lists some *in vitro* effects of HGH on isolated tissues from human subjects as well as experimental animals (9–14).

It is striking that HGH is a potent, fast acting lipolytic agent in rabbit fat cells but fails to produce a rapid stimulation of lipolysis in rat fat cells (12) as shown in Table III. The effect is very similar to that of ACTH in rabbit and rat fat cells and the glycoprotein hormones in rat fat cells; it is fast acting, glycerol release can be detected within 15 minutes, it is not blocked by inhibitors of protein synthesis, it requires the presence of Ca^{2+} ions, and it seems to be mediated by cyclic AMP.

The hormone possesses stimulatory effects in human leukemic lymphoblasts in culture (13). Although the concentration of HGH used did not significantly stimulate cell multiplication, perhaps because these cells proliferate rapidly, the hormone did stimulate synthesis of nucleic acids and proteins as judged by the incorporation of a labeled precursor (Table IV). These stimulatory effects were consistent at 0.01 and 0.1 μg/ml and most pronounced with cells exposed to HGH for 1.5 to 3.0 hours. Higher concentrations, e.g., 1.0 μg/ml, were less effective—and sometimes inhibitory. A recent report by Astaldi *et al.* (14) showed that as little as 10 ng/ml of the hormone incubated with normal human peripheral blood lymphocytes in cell culture for 5 days were able to stimulate lymphocyte blastogenesis.

II. Primary Structure

The amino acid sequence of HGH was first proposed in 1966 (15) and was followed by a detailed report including several small corrections (16).

Table IV—Effect of HGH on Human Leukemic Lymphoblasts in Culture[a]

Time/hour	DNA ([³H]thymidine)			RNA ([³H]uridine)			Protein ([³H]leucine)		
	0.01 μg/ml HGH	0.1 μg/ml HGH	1.0 μg/ml HGH	0.01 μg/ml HGH	0.1 μg/ml HGH	1.0 μg/ml HGH	0.01 μg/ml HGH	0.1 μg/ml HGH	1.0 μg/ml HGH
0.5	258	260	250	210	340	405	435	380	410
1.0	390	388	370	1025	1250	1350	870	980	1000
1.5	502	830	980	1520	2170	1745	1850	2840	2675
3.0	634	1235	1002	2030	4280	3460	2910	4430	3955
6.0	650	1020	1035	2120	3640	2980	3470	3850	3610

[a] Values in cpm/100 μg tissue.

HGH was then shown to consist of 188 amino acid residues with two disulfide bridges formed by residues 68–162 and 179–186, and a single tryptophan residue at position 25. In 1970, a comparison (17) of this structure with the recently complete structure of ovine prolactin (18) indicated that, although there was considerable homology between the two proteins, a peculiar realignment of a 13-residue segment in HGH was required to produce the best "fit" between the two structures. This segment ran from Ile-17 through Val-29 in the original HGH sequence, and contained the single tryptophan residue at position 25. At the time, it was felt that this reflected a genetic rearrangement rather than a potential error in sequencing (17). However, continuous comparisons with the emerging sequences of other closely related molecules, namely, human chorionic somatomammotropin (19) and sheep pituitary growth hormone (20), clearly indicated the possibility of an error in the location of the "tryptophan peptide" in the HGH sequence. A revised sequence for the amino-terminal portion of the molecule which provided conclusive evidence for the misplacement of the 15-residue segment from Ile-17 to Phe-31 was reported by Niall (21). Simultaneously, Li and Dixon (22) reported a revised overall sequence. This report included a repositioning of the 15-residue "tryptophan peptide" between positions 91 and 92 of the original sequence, along with an additional dipeptide (Leu-Arg) interposed immediately after the carboxyl-terminal residue of the "tryptophan peptide." A number of other small corrections were also noted in this report. This brought the total number of residues up to 190. A final revision published in 1972 (23) described the presently accepted primary structure of HGH as shown in Fig. 1. In this final form, HGH contains 191 amino acid residues, with the tryptophan appearing at position 86.

III. Secondary Structure

A. Optical Rotatory Dispersion

ORD measurements were made at 27° in solutions of a pH range from 1.3 to 12.9 (24). The α-helix contents calculated by two procedures (25,26) were in good agreement in all cases. In the absence of urea, a negative Cotton effect with a trough at 232–233 nm and a crossover point at 223–224 nm were found at each pH studied. The calculated α-helix contents are summarized in Table V. Both the unusually high α-helix content and its remarkable stability to alkaline treatment are evident in this data. Furthermore, it appears that only one-half the α-helix content of HGH is destroyed by treatment of the hormone with 8 *M* urea at pH 1.3. This

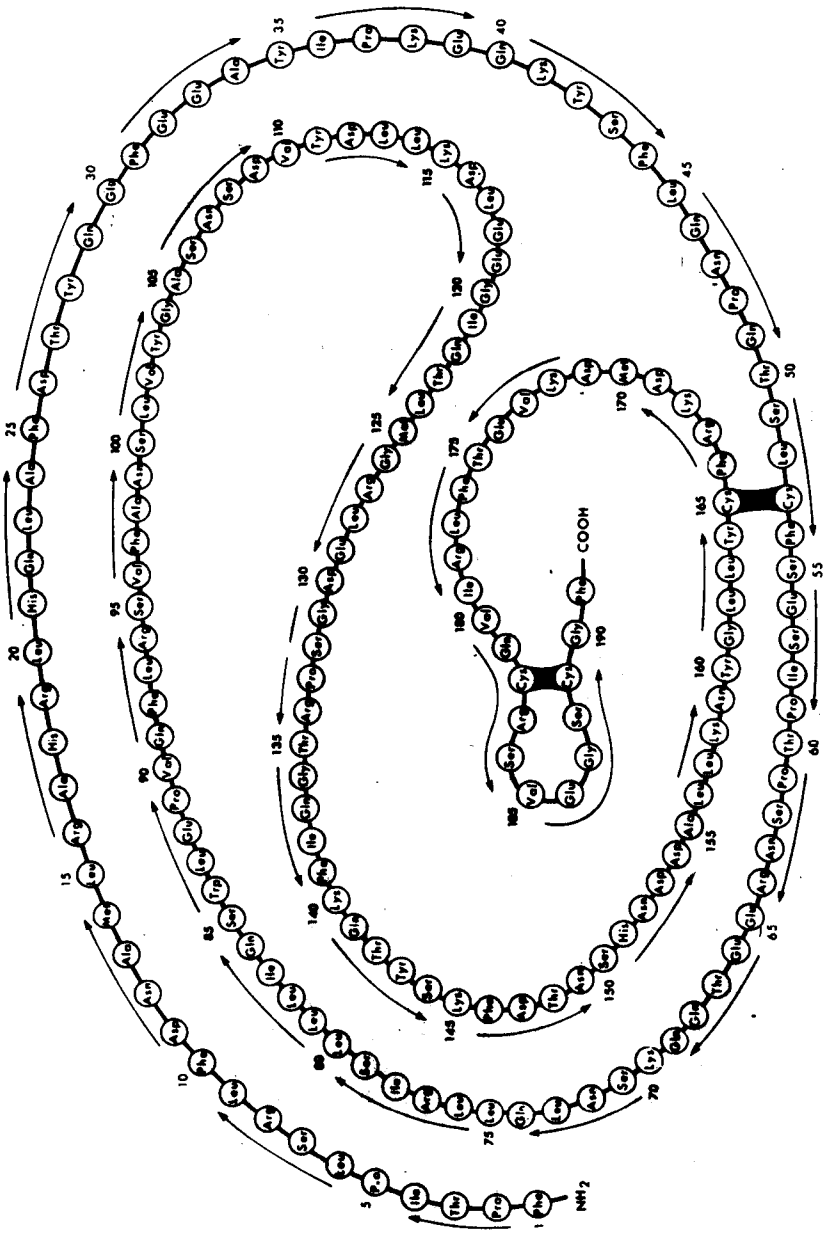

FIG. 1. Amino acid sequence of the HGH molecule.

Table V—α-Helix Contents Calculated from the Appropriate ORD Parameters for HGH[a]

Solvent pH	Schechter–Blout			Moffit–Yang	
	H_{226}	H_{193}	H_c	$\lambda_0 = 209$	$\lambda_0 = 212$
1.3	58	59	58	56	58
7.4	49	53	51	53	48
10.1	46	51	49	52	47
0.1 *M* KOH, pH ≈ 13	41	45	43	46	41
8 *M* urea, pH = 1.3	26	27	27	35	24

[a] Each value represents the average of two determinations.

report (24) also indicated that at pH 8.0, the secondary structure of HGH is essentially unaffected by urea at concentrations less than 4–5 *M*.

B. Circular Dichroism

The CD of HGH was found to exhibit two intense negative bands at 221 and 209 nm, both of which are characteristic of α-helical polypeptides (27–29). Figure 2 presents the CD spectra of HGH in the region of amide bond absorption and under a number of different solvent conditions (28). The mean residue molecular ellipticities and α-helix contents at pH 8.2 and 3.6 calculated from these spectra are shown in Table VI. These values are in excellent agreement with the α-helix contents previously reported from ORD measurements (24).

Aloj and Edelhoch (30) have also reported CD measurements on HGH and found α-helix contents of 50%–55%. These authors conclude that the secondary structure of HGH is virtually unchanged between pH 2.1 and 12.35. Significantly lower values of ellipticity and α-helix contents (~30%) have been reported by Sonenberg and Beychok (31). These authors also suggest the presence of 10–15% β-structure.

Besides indicating the extent of secondary structure, the data in Fig. 2 also demonstrate that the conformational changes produced by treating the HGH molecule with a number of perturbing solvents are all reversible on removal of the perturbants, with apparent reestablishment of the native structure. This reversibility even includes the extensive denaturation brought about by 5 *M* guanidine hydrochloride.

C. Deuterium and Tritium Exchange

In order to obtain a different type of information relating to the conformation of HGH in solution, deuterium exchange investigations have been

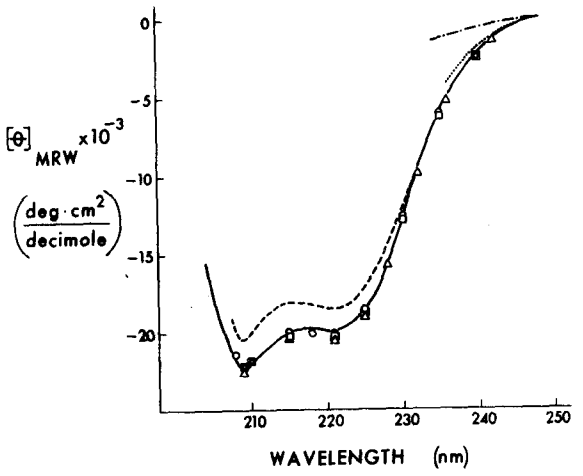

FIG. 2. Amide bond CD spectra of HGH in: 0.1 *M* Tris buffer, pH 8.2 (——); 0.1 *M* glycine hydrochloride buffer, pH 3.6 (----); 50% acetic acid (· · ·); and 5 *M* guanidine hydrochloride 0.1 *M* Tris buffer, pH 8.2 (— · —). Ellipticities at selected wavelengths following removal of the perturbing solvents by dialysis against 0.1 *M* Tris buffer, pH 8.2 are indicated by: glycine hydrochloride pH 3.6 (△); 50% acetic acid (○); and 5 *M* guanidine hydrochloride (□).

carried out (32). Approximately 50% of the hydrogens exchanged in 15 minutes at both pH 2.8 and 3.6, while complete exchange occurred at pH 9.3 in the same time period. An additional 20% of the hydrogens exchanged in 3 hours at pH values below 7.0. Apparently, the secondary amide hydrogens in HGH are very labile despite the fact that the optical data suggest approximately 50% of the peptide chain to be in an α-helical structure. Hydrogen–tritium exchange experiments have been reported by Cambiaso *et al.* (33). Even with a certain number of assumptions involved, their experiments show fair agreement with the deuterium exchange

Table VI—α-Helix Contents of HGH from CD Measurements

	$[\theta]_\lambda$[a]		α-Helix content (%) from $[\theta]_\lambda$	
pH	λ = 221 nm	λ = 209 nm	221 nm	209 mm
8.2	−19,700 ± 100	−21,800 ± 300	55	55
3.6	−18,500 ± 100	−20,500 ± 300	50	50

[a] Expressed as the mean ± S.E. (26,28).

data—at least for those portions of the tritium exchange which they tentatively assign to the secondary amide hydrogens.

IV. Tertiary Structure

A. Circular Dichroism

The CD spectrum of HGH (28) in the region of side-chain absorption is shown in Fig. 3. When dissolved in 0.1 *M* Tris-Cl buffer of pH 8.2, the spectrum exhibits a number of optically active bands between 325 and 250 nm; the two negative bands at 269 and 261 nm may be assigned to phenylalanine residues. The two negative bands at 283 and 277–278 nm are primarily due to tyrosine residues, although both tryptophan and disulfide bonds may contribute optical activity in this region. The positive asymmetric band between 320 and 288 nm is confidently assigned to the tryptophan residue(28,34).

Figure 3 also shows the effect of several perturbing solvents on these side-chain CD bands. Treatment with 5 *M* guanidine hydrochloride results in the loss of almost all the dichroism above 260 nm associated with the native protein, and only a broad, weakly negative band between 295 and 260 nm remaining. Glycine buffer (pH 3.6) results in a decrease in intensity of the positive tryptophan bands, with an apparent shift in the positive maximum from 294 to 296 nm. In this solvent, there are also four negative

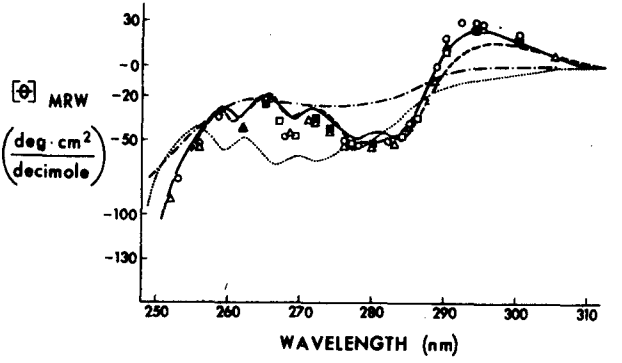

Fig. 3. Side-chain CD spectra of HGH in: 0.1 *M* Tris buffer, pH 8.2 (——); 0.1 *M* glycine hydrochloride buffer, pH 3.6 (---); 50% acetic acid (· · ·); and 5 *M* guanidine hydrochloride 0.1 *M* Tris buffer, pH 8.2 (—·—·). Ellipticities at selected wavelengths following removal of the perturbing solvents by dialysis against 0.1 *M* Tris buffer, pH 8.2, are indicated by: glycine hydrochloride, pH 3.6 (△); 50% acetic acid (○); and 5 *M* guanidine hydrochloride (□).