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Human somatotropin: Biological characterization of the recombinant molecule*

(human growth hormone/ornithine decarboxylase/mammary gland/radioimmunoassay)

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ABSTRACT **The recombinant hormone obtained by noncovalent interaction of the NH_2-terminal 134 amino acid fragment with the COOH-terminal 51 amino acid fragment of the reduced-carbamidomethylated human somatotropin molecule is found to exhibit nearly full biological activity of the native hormone, as evidenced by the stimulation of hepatic ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) *in vivo* and protein synthesis in mouse mammary gland *in vitro*. Radioimmunoassay data indicate that the recombinant behaves immunochemically in a manner almost identical to that of the native hormone.**

It has been reported that hydrolysis of human somatotropin (HGH) with human plasmin does not cause any changes in the biological properties of the hormone (1–5). The predominant action of plasmin on HGH is the cleavage of the Arg-Thr and Lys-Gln bonds at positions 134–135 and 140–141, respectively (see Fig. 1). Thus, the main product of a limited plasmin digestion of the hormone is composed of the NH_2-terminal portion (residues 1–134) and the COOH-terminal portion (residues 141–191) of HGH. These are connected to each other by a disulfide linkage at sequence positions 53 and 165. The removal of the hexapeptide (residues 135–140) from the structure of HGH does not alter the conformation of the molecule as evidenced by circular dichroism spectra (6).

When plasmin-modified HGH is submitted to reduction and alkylation, two peptide fragments are separated by exclusion chromatography on Sephadex G-50 in 10% (wt/vol) acetic acid: [Cys(Cam)^{53}-HGH-(1–134)] and [Cys(Cam)^{165,182,189}-HGH-(141–191)] (Cam is carbamidomethyl). Bioassay data showed both fragments to be biologically active (4). The biological activity of the NH_2-terminal 1-134 fragment is about 14% of that for native HGH by both the rat tibia and pigeon crop-sac tests. The COOH-terminal 141-191 fragment also exhibits activity in both tests, although it is less active than the NH_2-terminal fragment. Complement fixation and radioimmunoassay experiments showed that the NH_2-terminal fragment is also more immunoreactive (7). We reported recently the restoration of full rat tibia and pigeon crop-sac stimulating activities by noncovalent interaction of these two fragments of HGH (8). This communication describes biological and immunochemical properties of the recombinant HGH molecule and supports the conclusion that the recombinant possesses full or nearly full HGH activity.

MATERIALS AND METHODS

Human somatotropin was isolated from fresh-frozen pituitary glands as previously described (9). Cys(Cam)^{53}-HGH-(1-134) and Cys(Cam)^{165,182,189}-HGH-(141-191) were prepared from plasmin digests of HGH as previously described (4) except that the NH_2-terminal fragment was further purified by gel filtration on Sephadex G-100 in 0.01 M NH_4HCO_3 of pH 8.2. The recombinant HGH was prepared by the procedure recently published (8). DL-[^{14}C]ornithine monohydrochloride (7.6 mCi/mmol) and [^{3}H]leucine (5.0 Ci/mmol) were obtained from New England Nuclear Corp.

For radioimmunoassay, the double-antibody procedure of Schalch and Reichlin (10) was used with slight modification, employing a guinea pig antiserum to native HGH. Highly purified HGH (9) was used for the preparation of standards and labeling with ^{131}I according to the procedure of Greenwood *et al.* (11). The hepatic ornithine decarboxylase activity (L-ornithine carboxy-lyase, EC 4.1.1.17) was assayed by the procedure

Table 1. Stimulation of hepatic ornithine decarboxylase activity by the recombinant obtained by noncovalent interaction of the two fragments of HGH

Preparation	Dose (nmol)	Ornithine decarboxylase activity*
Saline	0.0	16.5 ± 6.6
HGH	0.045	60.8 ± 15.4
	0.135	126.8 ± 8.3
Recombinant †	0.047	21.0 ± 5.9
	0.141	113.2 ± 16.0

* Values (pmol of $^{14}CO_2$ released per mg of protein per hr) in mean ± SEM. Four rats were in each group.
† Potency relative to HGH was 69% with 95% confidence limit of 43–98% and $\lambda = 0.15$.

Table 2. Stimulation of protein synthesis in mouse mammary gland *in vitro* by the recombinant obtained by noncovalent interaction of the two fragments of HGH

Preparation	Concentration* (g/ml)	[^{3}H]Leucine incorporation † (cpm/mg of protein) ($\times 10^{-4}$)
Control	0	1.88 ± 0.30
HGH	22.5	3.34 ± 0.34
	67.5	5.44 ± 0.31
Recombinant‡	22.5	3.63 ± 0.27
	67.5	5.07 ± 0.35

* Three daily treatments; concentrations are final concentrations.
† Mean ± SEM from five replicates.
‡ Potency relative to HGH, 97% with 95% confidence limit of 60–116% and $\lambda = 0.20$.

Abbreviation: HGH, human somatotropin (growth hormone); Cam, carbamidomethyl.

* This is paper no. 50 in a series. Paper no. 49 is ref. 15.

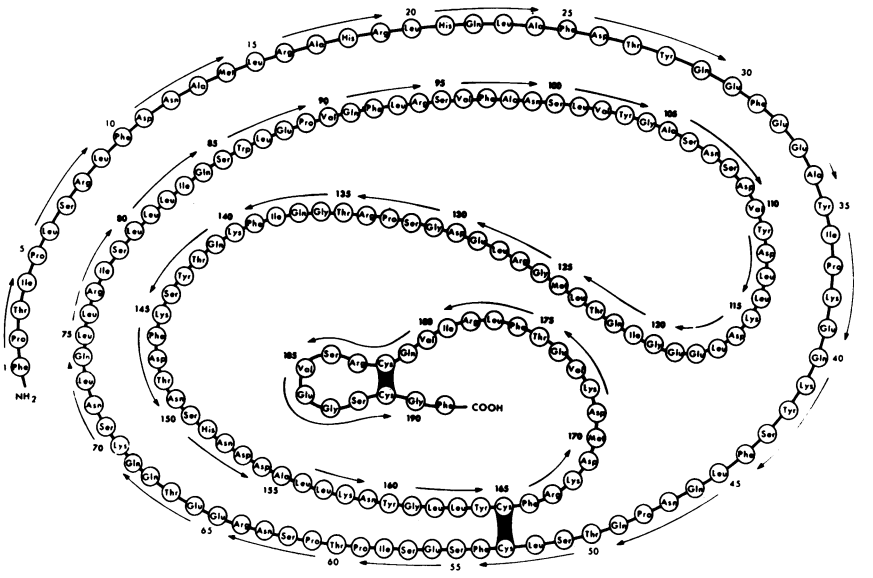

FIG. 1. Amino acid sequence of the HGH molecule.

of Jänne and Williams-Ashman (12) in male rats, 21 days of age, of the Sprague-Dawley strain. Prolactin activity was estimated by using a modification of the mammary gland bioassay previously described (13). In this bioassay, prolactins specifically stimulate the incorporation of tritium-labeled leucine into trichloroacetic-acid-precipitable proteins in organ-cultured mammary tissues of mid-pregnant BALB/c mice.

RESULTS AND DISCUSSION

Table 1 summarizes the results of the ornithine-decarboxylase-stimulating activity of the recombinant. In comparison with the activity of the native hormone, the recombinant possesses 69% of HGH potency. Earlier studies (14) showed that the NH_2-terminal fragment had only 10% potency of the native hormone and the COOH-terminal fragment was inactive at the highest dose tested (120 nmol).

It may be seen in Table 2 that the recombinant had 97% potency of HGH in stimulating protein synthesis in mouse mammary gland *in vitro*. This is in agreement with the earlier experiment (8) in which the recombinant exhibits full prolactin activity of HGH as assayed by the pigeon crop-sac method.

Figure 2 presents the radioimmunoassay results with un-

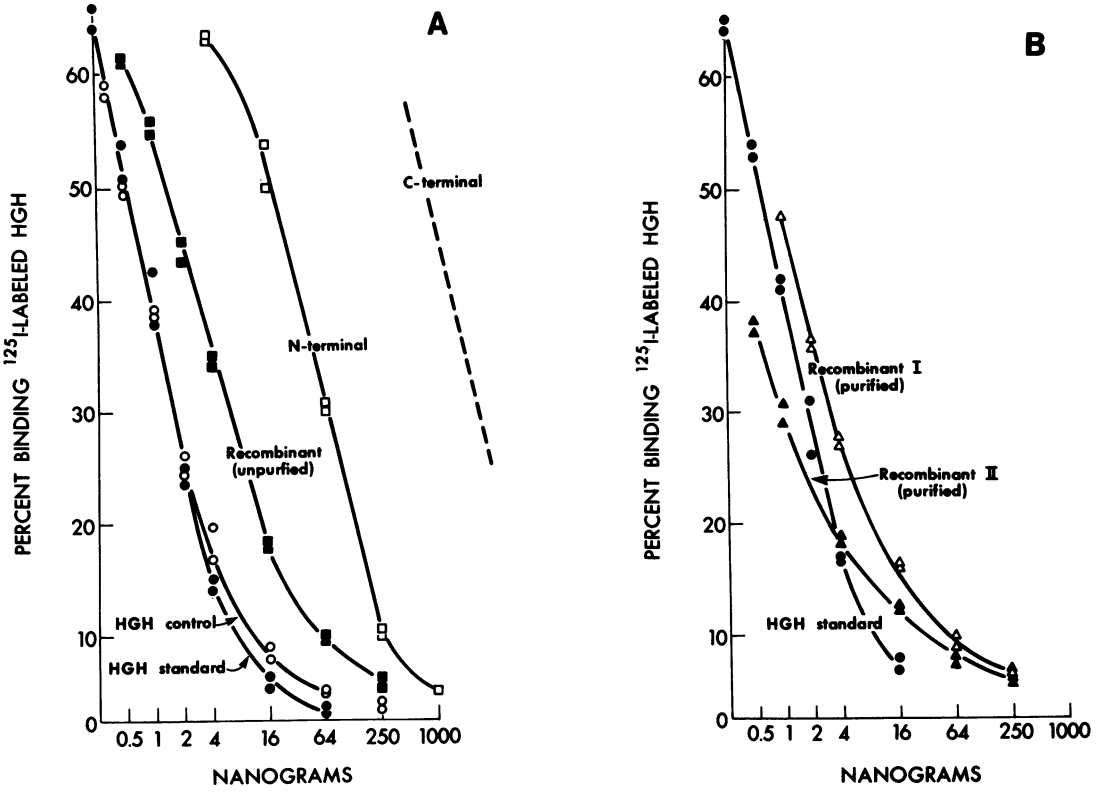

FIG. 2. Competition of the recombinant and human somatotropin in the HGH radioimmunoassay system. Final dilution of guinea pig antiserum was 1/140,000.

purified and purified recombinants using guinea pig antiserum. It may be noted that the unpurified recombinant showed much stronger inhibition than that obtained with the NH_2-terminal fragment (Fig. 2A). After purification, the recombinant gave inhibition curves which were nearly identical to that of HGH standard (Fig. 2B). There is a slight lack of parallelism shown by the slope of inhibition of the purified or non-purified recombinant and that of HGH. This may be due to the fact that the recombinant is missing the hexapeptide Thr-Gly-Gln-Ile-Phe-Lys from residues 135-140 (see Fig. 1) and the two disulfide bridges. It may also be noted that the HGH control (Fig. 2A) showed no detectable effect on the immunoreactivity of native HGH subjected to the same conditions of storage undergone by the fragments during the formation of the recombinant. It was fortuitous that the conditions which facilitated the formation of the recombinant such as the pH (8.4) and the temperature (4°) were the same conditions utilized in the radioimmunoassay, thus minimizing or preventing the dissociation of the recombinant during the assay.

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