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HUMAN SOMATOTROPIN: SEMISYNTHESIS OF THE HORMONE BY NONCOVALENT INTERACTION OF THE NH_2-TERMINAL FRAGMENT WITH SYNTHETIC ANALOGS OF THE COOH-TERMINAL FRAGMENT*

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SUMMARY: Complementation of the natural NH_2-terminal fragment (consisting of 134 amino acid residues) with synthetic analogs of COOH-terminal fragments of 52 or 47 amino acids of reduced-carbamidomethylated human somatotropin gave recombinants with full growth-promoting activity as evidenced by the tibia test. Radioimmunoassay data show that the semisynthetic recombinant hormones possess nearly full immunoreactivity as compared to the native molecule.

The cleavage of human somatotropin (HGH, Fig. 1) by limited plasmin digestion and reduced-carbamidomethylation into an NH_2-terminal fragment of 134 amino acids and COOH-terminal fragment of 51 amino acids has recently been described (1). Restoration of full biological activity by noncovalent interaction of the NH_2-terminal fragment with the natural COOH-terminal fragment (2,3) or a synthetic COOH-terminal fragment of 52 amino acids (4) has also been described. We report here complementation experiments using the natural [Cys(Cam)^{53}]-HGH-(1-134) with synthetic [Nle^{170}, Ala^{165,182,189}]-HGH-(140-191) or [Nle^{170}, Ala^{165,182,189}]-HGH-(145-191) to obtain recombinant hormones with full growth-promoting activity and nearly full immunoreactivity.

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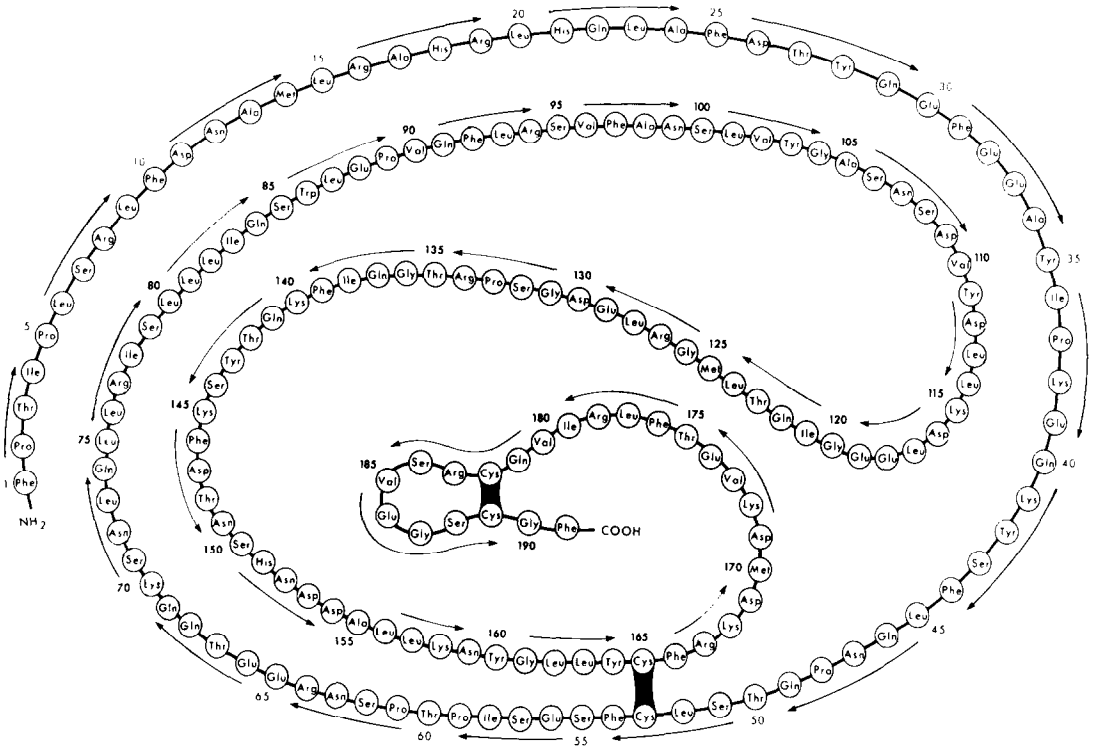

Fig. 1. The amino acid sequence of the HGH molecule.

MATERIALS AND METHODS

Human somatotropin was isolated from fresh-frozen pituitary glands as previously described (5). Plasmic digests of HGH and the isolation of [Cys(Cam)^{53}]-HGH-(1-134) were obtained as described by Li and Graf (1). The NH_2-terminal fragment was further purified by gel-filtration on Sephadex G-100 in 0.01M NH_4HCO_3 at pH 8.2 (6). Analogs of the COOH-terminal fragments, [Nle^{170}, Ala^{165,182,189}]-HGH-(140-191) and [Nle^{170},Ala^{165,182,189}]-HGH-(145-191) were synthesized as described (7). The complementation experiments were carried out by published procedures (2,8). Exclusion chromatography of reaction mixtures was performed at 22° on a Sephadex G-100 column (1.5 x 60 cm) in 0.01M NH_4HCO_3 at pH 8.2.

For radioimmunoassay, the double-antibody procedure (9) was used with slight modification using a guinea pig antiserum against HGH. The growth-promoting activity was estimated by the rat tibia test (10).

RESULTS

Complementation reactions were performed using 0.43 nmole

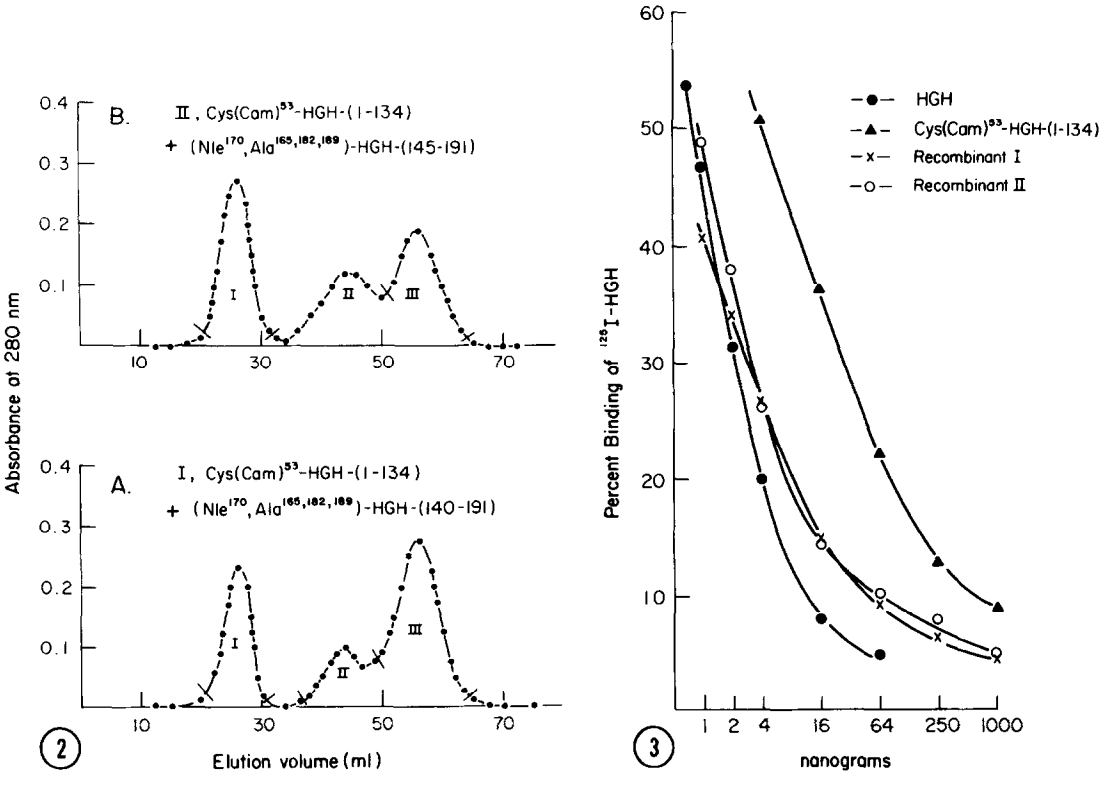

Fig. 2. Exclusion chromatography of the reaction mixture on a Sephadex G-100 column (1.5 x 60cm) in 0.01M NH_4HCO_3 at pH 8.2.

Fig. 3. Immunoreactivity of recombinant hormones relative to the native HGH standard and [Cys(Cam)^{53}]-HGH-(1-134). Final dilution of guinea pig antiserum to HGH was 1/100,000.

each of the NH_2-terminal fragment and the synthetic analog of the COOH-terminal fragment as described (8). Fig. 2 shows the elution pattern of the reaction mixture on Sephadex G-100. It may be noted that approximately 45% of the material appears in peak III at a Ve/Vo ratio of 2.12 for recombinant I and 30% for recombinant II. The elution positions of the two recombinants are the same for HGH under identical experimental conditions. The recovered protein from peak III was assayed for growth-promoting activity and the results are summarized in Table 1.

Table 1

Growth-Promoting Activity of Recombinant Hormones by Rat Tibia Assay

Preparation	Total dose (μg)	Response[a]
HGH	20	245.0 ± 9.5
	60	290.2 ± 2.9
Recombinant I[b, d]	20	249.2 ± 9.2
	60	286.0 ± 2.1
Recombinant II[c, e]	20	248.5 ± 7.7
	60	297.2 ±10.0
Saline	0	167.0 ± 1.3

[a]Tibia width in micrometers: mean ± SEM; four animals in each group.

[b]Recombinant I: [Cys(Cam)^{53}]-HGH-(1-134)+[Nle^{170}, Ala$^{165, 182, 189}$]-HGH-(140-191).

[c]Recombinant II: [Cys(Cam)^{53}]-HGH-(1-134)+[Nle^{170}, Ala$^{165, 182, 189}$]-HGH-(145-191).

[d]Relative potency to HGH, 100%, with 95% confidence limits of 65-154 and λ = 0.16.

[e]Relative potency to HGH, 113%, with 95% confidence limits of 74-180 and λ = 0.16.

It is evident that both recombinants exhibit full somatotropic activity in comparison with the native hormone.

Radioimmunoassay of the recombinants showed a strong cross-reaction which was almost identical to that of the native HGH standard at low concentrations whereas the NH_2-terminal [Cys(Cam)^{53}]-HGH-(1-134) gave only a slight cross-reaction.

DISCUSSION

Earlier studies showed that [Cys(Cam)^{53}]-HGH-(1-134)

possesses only 14% of HGH potency (1,11) and the synthetic analogs of the COOH-terminal fragment are even less active (7). Combinations of these two fragments in experiments herein described regenerate the full growth-promoting potency of the hormone as shown in Table 1. The present experiments show that substitution of Met-170 with Nle and of Cys(Cam)-165, 182, 189 with Ala in the COOH-terminal fragment does not affect the complementation reaction or the properties of the recombinant hormone. In addition, elimination of 5 amino acid residues at the NH_2-terminus of the COOH-terminal fragment gives rise to a recombinant HGH molecule with full growth-promoting activity and nearly full immunoreactivity (Table 1, Figs. 2 and 3). The number of additional amino acid residues that can be eliminated without affecting the complementation reaction remains to be determined.

The radioimmunoassay results with recombinant hormones gave inhibition curves which were nearly identical to that of HGH (see Fig. 3). The origin of lack of parallelism shown by recombinants is presently unknown. The lack of parallelism was also observed when the NH_2-terminal fragment complemented with the synthetic [Cys(Cam)^{165,182,189}]-HGH-(140-191) (4).

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