Noncovalent interaction of the NH$_2$-terminal fragment of human somatotropin with the COOH-terminal fragment of human choriomammotropin to generate growth-promoting activity*

(human growth hormone/tibia test/radioimmunoassay/human placenta lactogen)

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ABSTRACT Complementations of the plasmin fragments of reduced-carbamoylmethylated (Cam) human somatotropin (hGH) with those of reduced-carbamoylmethylated human chorionic somatomammotropin (hCS) have been investigated. It was found that the recombinant obtained by noncovalent interaction of [Cys(Cam)$_{53}$]hCS-(1-133) with [Cys(Cam)$_{165,182,189}$]hCS-(141-191) exhibits 50% growth-promoting activity and nearly full immunoreactivity. Complementation of [Cys-(Cam)$_{53}$]hCS-(1-133) with the COOH-terminal fragment of hGH generated lower growth-promoting and immunological activities.

The NH$_2$-terminal 134-amino-acid fragment of the reduced carbamoylmethylated (Cam) human somatotropin (human growth hormone, hGH) molecule has been shown to react noncovalently with the natural or synthetic COOH-terminal 51-amino-acid fragment to restore full biological and immunological activity (2-4). Because the primary structures of human chorionic somatomammotropin (hCS) (5) and hGH (6) are nearly identical (see Fig. 1), it seems of interest to investigate the possibility of generating biological activities by complementation of plasmin fragments of hGH with those of hCS.

MATERIALS AND METHODS

Plasmin fragments of reduced-carbamoylmethylated hGH and hCS were obtained as previously described (7,8). Exclusion chromatography of the reaction mixture was performed at 22$^\circ$C on a Sephadex G-100 column (1.5 x 60 cm) in 0.01 M NH$_4$HCO$_3$ at pH 8.4. For radioimmunoassay, the double antibody procedure (9) using a guinea pig antiserum to native hGH was followed. The growth-promoting activity was determined by the rat tibia test (10).

RESULTS

Complementation reactions were carried out by adding 6.7 mg (0.43 $\mu$mol) of [Cys(Cam)$_{53}$]hGH-(1-134) or [Cys(Cam)$_{53}$]-hCS-(1-133) in 4.9 ml of Tris-HCl buffer at pH 8.4 [0.1 M, 5% (vol/vol) butanolo to 2.7 mg (0.44 $\mu$mol) of [Cys(Cam)$_{165,182,189}$]hCS-(141-191) or [Cys(Cam)$_{165,182,189}$]hGH-(141-191) in 0.1 ml of 1 M NH$_4$OH. Both solutions were clear before mixing and became slightly turbid after mixing. The cloudy solution was kept at 22$^\circ$C for 3-5 hr and then stored in the refrigerator (2$^\circ$C) for at least 10 days. After the removal of some insoluble material, the clear reaction mixture was submitted to exclusion chromatography on Sephadex G-100; elution patterns are shown in Fig. 2. Approximately 45% of the protein appeared in a distinct peak (designated fraction III) for recombinant I and 30% for recombinant II with a relative elution volume $V_e/V_o$ of 1.85. This is exactly the elution position of native hGH or hCS under the same experimental conditions.

The growth-promoting activity of purified recombinants I and II were summarized in Table I. It is evident that recombinant I had 50% of the potency of hGH, whereas recombinant II was considerably less active.

Fig. 3 presents the radioimmunoassay results with purified recombinants using guinea pig antiserum to hGH. It may be noted that recombinant I at low concentrations gave an inhibition nearly identical to that of hGH, but the inhibition curve was not parallel with that for hGH. Although recombinant II gave a parallel inhibition curve, it was much less effective than hGH, requiring at least 30 times higher antigen concentrations to give the same degree of inhibition.

DISCUSSION

Complementation reactions have been slightly modified from earlier experiments (2,4) in that the COOH-terminal fragment was first dissolved in 1 M NH$_4$OH to give a clear solution before mixing with the NH$_2$-terminal fragment. This gives rise to a better yield of purified recombinant product. For complementation of the hGH fragments, the yield has been increased from 23% (2) to nearly 50% (data not shown).

As shown in Table I and Fig. 3, [Cys(Cam)$_{53}$]hGH-(1-134) is capable of reacting noncovalently with [Cys(Cam)$_{165,182,189}$]hCS-(141-191) and generating a recombinant with significant growth-promoting activity and nearly full immunoreactivity against the antiserum to hGH.

Both hGH and hCS molecules consist of 191 amino acids with two disulfide bridges and a single tryptophan residue; these structures are located in the same positions in the two molecules (see Fig. 1). The differences in the amino acid sequences of hCS and hGH occur chiefly in the first 133 residues (26 positions); only two residues (at positions 153 and 179) differ in the 134-191 COOH-terminal sequence. In addition, the two plasmin fragments of reduced-carbamoylmethylated hGH exhibit some hormonal (7) and immunological (11) activities, whereas the two fragments from hCS are not active (8). It is not surprising that the [Cys(Cam)$_{53}$]hCS-(1-133) fragment does complement significantly with the COOH-terminal fragment.

Abbreviations: hGH, human somatotropin (growth hormone); hCS, human chorionic somatomammotropin (human choriomammotropin or human placental lactogen); Cam, carbamoylmethyl; recombinant I, purified product obtained by complementation of [Cys(Cam)$_{53}$]-hGH-(1-134) with [Cys(Cam)$_{165,182,189}$]hCS-(141-191); recombinant II, purified product obtained by complementation of [Cys(Cam)$_{53}$]-hCS-(1-133) with [Cys(Cam)$_{165,182,189}$]hGH-(141-191).

* This is paper no. 54 in the human somatotropin series. Paper no. 53 is ref. 1.
FIG. 1. Primary structures of hGH (Upper) and hCS (Lower).
of hGH as indicated by exclusion chromatography (see Fig. 2), and the recombinant has measurable hormonal and immunological activities (see Table 1 and Fig. 3). However, the NH$_2$-terminal fragment of hGH reacts noncovalently with [Cys(Cam)$_{165,182,189}$]hCS-(141-191) to generate only 50% of the growth-promoting activity of hGH (see Table 1). It is possible that substitutions of Asp$^{153}$ and Ile$^{178}$ by His and Met, respectively, in the hCS structure prevent the generation of full biological activity. These results demonstrate that the three-di-

![Fig. 2](image-url)

**Fig. 2.** Gel filtration of the fragment mixtures on a Sephadex G-100 column (1.5 X 60 cm) in 0.01 M NH$_4$HCO$_3$ buffer at pH 8.4. (A) Mixture of [Cys(Cam)$_{53}$]hGH-(1-134) and [Cys(Cam)$_{165,182,189}$]hCS-(141-191). Forerun, 7 ml. (B) Mixture of [Cys(Cam)$_{53}$]hCS-(1-133) and [Cys(Cam)$_{165,182,189}$]hGH-(141-191). Forerun, 27 ml.

![Fig. 3](image-url)

**Fig. 3.** Competition of the recombinants and hGH in the hGH radiolmmunoassay system. Final dilution of guinea pig antiserum was 1/100,000. $\bullet$, hGH; $\circ$, [Cys(Cam)$_{53}$]hGH-(1-134) plus [Cys(Cam)$_{165,182,189}$]hCS-(141-191); $\Box$, [Cys(Cam)$_{53}$]hGH-(1-134); $\bigcirc$, [Cys(Cam)$_{53}$]hCS-(1-133) plus [Cys(Cam)$_{165,182,189}$]hGH-(141-191); $\Delta$, [Cys(Cam)$_{165,182,189}$]hGH-(141-191); $\bigtriangleup$, [Cys(Cam)$_{165,182,189}$]hCS-(1-133); $\blacksquare$, [Cys(Cam)$_{165,182,189}$]hCS-(141-191).

The dimensional structures of [Cys(Cam)$_{53}$]hGH-(1-134) and [Cys(Cam)$_{53}$]hCS-(1-133) are not equivalent in spite of the fact that these two fragments have high $\alpha$-helical content (2, 8).

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Table 1. Growth-promoting activity of the recombinant hormones by rat tibia assay

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Total dose, µg</th>
<th>Response*</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGH</td>
<td>20</td>
<td>242.8 ± 3.8</td>
</tr>
<tr>
<td>Recombinant I$^1$</td>
<td>60</td>
<td>282.8 ± 1.5</td>
</tr>
<tr>
<td>Recombinant II$^1$</td>
<td>60</td>
<td>212.0 ± 4.7</td>
</tr>
<tr>
<td>[Cys(Cam)$_{53}$]hGH-(1-134)</td>
<td>50</td>
<td>259.8 ± 2.9</td>
</tr>
<tr>
<td>[Cys(Cam)$_{53}$]hCS-(1-133)</td>
<td>30</td>
<td>218.5 ± 6.8</td>
</tr>
<tr>
<td>Saline</td>
<td>90</td>
<td>229.0 ± 4.1</td>
</tr>
</tbody>
</table>

* Tibia width in µm; mean ± SEM; four animals in each group.
$^1$ Recombinant I: [Cys(Cam)$_{53}$]hGH-(1-134) plus [Cys(Cam)$_{165,182,189}$]hCS-(141-191).
$^2$ Potency relative to hGH, 50%, with 95% confidence limit of 39-62% and $\lambda = 0.08$.
$^3$ Potency relative to [Cys(Cam)$_{53}$]hGH-(1-134), 491% with 95% confidence limit of 398-592% and $\lambda = 0.07$.
$^4$ Recombinant II: [Cys(Cam)$_{53}$]hCS-(1-133) plus [Cys(Cam)$_{165,182,189}$]hGH-(141-191); the dose-response curve is not parallel with that of hGH and the relative potency cannot be calculated.
$^5$ Potency relative to hGH, 10%, with 95% confidence limit of 9-12% and $\lambda = 0.07$. 