Involvement of lysine residues in the binding of hGH and bGH to somatotropic receptors

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The biological activities of human (hGH) and bovine (bGH) growth hormone derivatives obtained by chemical modification of the lysine residues were studied by radioreceptor assays using rabbit liver homogenates for somatotropic activity (SA). Control treatment with BH_4^- had a very slight effect on the SA, whereas the methylation and ethylation drastically reduced the acitivty of both hormones. Guanidination of these hormones and even acetimidination at a lower rate are accompanied by a considerable loss of biological activity. These results show the involvement of lysine residues in the interaction of hGH and bGH with somatotropic receptors. The structure-function relationship of these molecules is discussed, suggesting that the lysine or arginine residues in positions 41, 64, 70 and 115 might be particularly implicated.

Human growth hormone *Bovine growth hormone* *Human chorionic somatomammotropin* *Lysine residue* *Receptor*

1. INTRODUCTION

Though highly purified preparations of bGH and hGH have been available for several years, our knowledge on the relationship between the chemical structure and biological activity of growth hormones is limited. We know that the biological activity is preserved after reduction of the disulfide bonds if the -SH groups are blocked by iodoacetamide. Modification of the tryptophan or some tyrosine residues, oxidation of the methionines can be achieved without or with only a partial loss of the growth-promoting activity [1–4]. Using enzymic cleavage and association of the NH_2-terminal hGH fragment 1–134 with synthetic COOH-terminal fragments it was shown that full promoting activity can be obtained when fragment 1–134 is recombined with a 140–191 peptide with very similar sequence to that of the natural hGH. The NH_2-terminal segment 1–134 alone exhibited only 14% of the biological activity [1,5–7].

The fact that a bacterially synthesized methionyl hGH is fully active suggests that the α-NH_2 group of the N-terminal Phe can be modified without loss of biological activity [8]. Nothing is known about the intervention of the ϵ-NH_2 in the growth-promoting activity of these hormones. We have studied the effect of a more or less profound modification of these groups on the biological activity. Only chemical modifications preserving the basicity of the group (methylation, ethylation, guanidination and acetimidination) were achieved, since the abolition of positive charges can cause secondary effects on the conformation of the protein. The biological activity was investigated using a radioligand receptor assay with rabbit liver homogenates.

2. MATERIALS AND METHODS

2.1. *Chemical modifications*

bGH was a highly purified preparation purchased from Pentex (Miles) (1 IU/mg) and hGH was a gift from Dr Dray (Institut Pasteur, Paris) (1 IU/mg).

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2.1.1. Reductive alkylation

Reductive methylation and ethylation were carried out by addition of sodium borohydride and formaldehyde or acetaldehyde to the hormone (0.5–1.0 mg) dissolved in borate buffer (pH 9) as in [9]. The solutions were dialyzed against pyridine solution (1%) and freeze-dried. Determinations of lysine, methyl- and ethyllysine were made using a Technicon autoanalyzer and elution gradients containing isopropanol [9,10]. A blank for biological assays was prepared by treating hormone with borohydride and borate buffer (pH 9, no added aldehyde).

2.1.2. Guanidination

This reaction was performed using 0.3 M *O*-methylisourea sulfate (Aldrich, Europe) at pH 10.3 and 5°C (0.7 mg GH/0.15 ml) for 24 h. At the end of the reaction, the solutions were dialyzed against pyridine solutions. After 1 day of reaction, the solutions became slightly opalescent. In both cases, the solutions were centrifuged and the precipitate discarded. The degree of guanidination was measured by amino acid analysis. A blank was prepared by treatment of the hormone at pH 10.4 for 5 days at 5°C.

2.1.3. Acetimidination

To obtain a low degree of chemical modification, this reaction was performed at a low concentration of reagent (ethylacetimidate hydrochloride, Aldrich) for 24 h, at pH 10 and 5°C. Determination of the ϵ-acetimidyllysine was performed by amino acid analysis as in [11].

2.2. *Radioreceptor assay of growth-promoting activity*

This activity was determined by radioreceptor assay [12] with membranes of rabbit liver on day 17 of pregnancy in the presence of ^{125}I-labelled Pentex bovine growth hormone (bGH) and standard unlabelled bGH (Pentex, 1 IU/mg). In rabbit, the liver is particularly rich in GH receptors and poor in prolactin receptors. Each sample was tested several times in duplicate at various dilutions in the following buffer: 25 mM Tris (pH 7.6), 10 mM $MgCl_2$, bovine serum albumin 0.1% (w/v).

3. RESULTS AND DISCUSSION

The degree of modification as determined by amino acid analysis is shown in table 1 as percentage and number of modified residues out of the total number. As usual the degree of ethylation is somewhat lower than that of methylation. The more disturbing action of the ethyl group can be compensated by a lower degree of modification. The degree of guanidination as well as that of alkylation was very similar in both hormones.

The results obtained in the radioreceptor assay for somatotropic activity are reported in figs 1 and 2. Table 2 lists the relative potencies expressed in terms of native hormone (percentage). These values were calculated by comparison of the hormone concentration needed for 50% inhibition of specific binding.

As shown in table 2, the reductive action of borohydride alone has a very slight effect on the biological activity (perhaps as the result of partial reduction and disordered reconstitution of disulfide bonds as we have observed in the case of ovine prolactin; unpublished). The effect of methylation or ethylation in reductive conditions is much more clear-cut: 50–80% of the activity is lost. Guanidination or limited acetimidination lead to similar results for hGH but with much more

Table 1

Degree of modification of the lysine residues

Modified hormones	Total no.of lysine residues	Methylation		Ethylation		Guanidination		Acetimidination	
		%	Number	%	Number	%	Number	%	Number
bGH	11	69	7	45	4–5	66	7	10–20	1–2
hGH	9	74	7	52	5	52	5	10–20	1–2

Values denote % and number of modified lysines/molecule

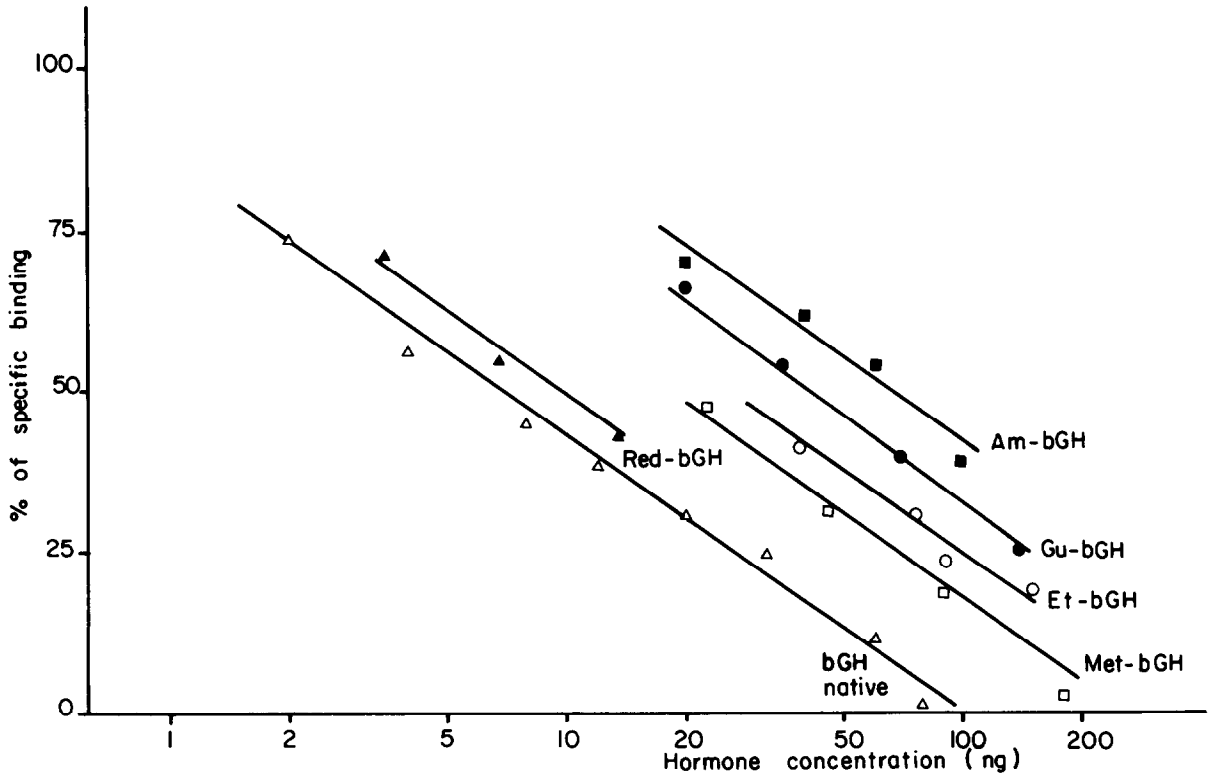

Fig.1. Specific binding of native and modified bGH in a radioreceptor assay for somatotropic activity with pregnant rabbit liver membranes. The GH activity is expressed in bovine growth hormone equivalents (bGH Pentex, 1 IU/mg) (△—△); Red-bGH, reduced bGH (▲—▲); Met-bGH, methylated bGH (□—□); Am-bGH, acetimidinated bGH (■—■); Et-bGH, ethylated bGH (○—○); Gu-bGH, guanidinated bGH (●—●).

drastic loss of activity in the case of bGH. In another bioassay (the hypophysectomized rat body weight test) Blumgrund de Satz and Santomé [13] did not observe a considerable loss of biological activity in the case of the amidinated bGH. In our test, however, all the lysine-modified derivatives exhibit a significant loss of activity when compared with the blanks. Examination of the amino acid sequences of different molecules with growth-promoting activity shows (see fig.3) the conservation of lysine (or arginine) residues at positions (of the hGH sequence) 41, 64, 70, 115, 145, 158 and 168 [14] suggesting that they might play a role in the biological activity. In contrast, Lys 29 of bGH

Table 2

Biological activity of modified growth hormones (in percentage of native hormone)[a]

Hormone	BH_4^-, pH 9 (blank)	Methyl-ated	Ethyl-ated	pH 10 (blank)	Guanidin-ated	Acetimidin-ated
bGH	71	39	27	100	17	11
hGH	88	13	39	100	58	51

[a] Relative potencies expressed as a percentage of the binding activity of native oCS calculated by comparison of the hormone concentration needed for 50% inhibition of specific binding

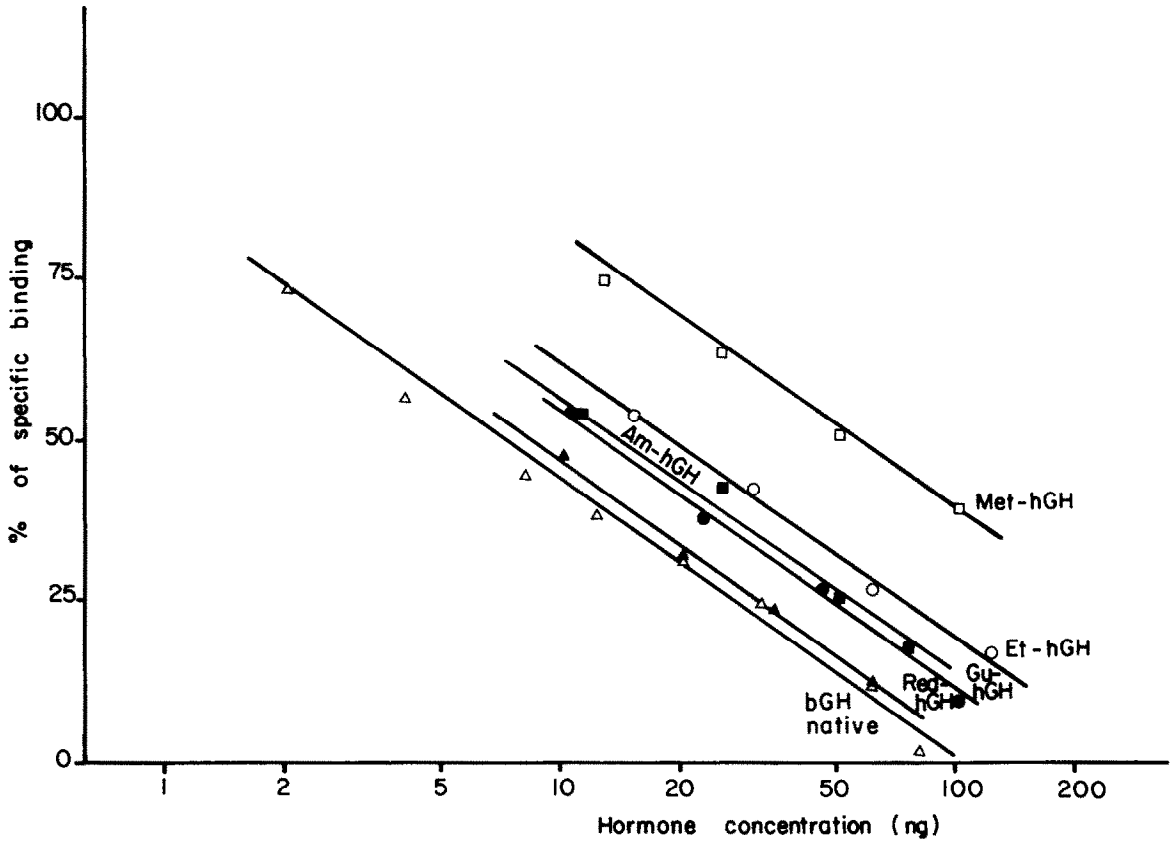

Fig.2. Specific binding of native bGH and modified hGH in a radioreceptor assay for somatotropic activity with pregnant rabbit liver membranes. The GH activity is expressed in bovine growth hormone equivalents (bGH Pentex, 1 IU/mg) (△—△); Red-hGH, reduced hGH (▲—▲); Met-hGH, methylated hGH (□—□); Am-hGH, acetimidinated hGH (■—■); Et-hGH, ethylated hGH (○—○); Gu-hGH, guanidinated hGH (○—○).

is replaced by a glutamine in hGH and Lys 38 of hGH by glutamic acid in bGH, a clear indication of the non-essential character of these basic residues. Recently, peptide segments 1–43 and 32–46 of hGH have been synthesized. These peptides which contained Lys 41 show some biological potency [15] when assayed in an in vitro test based on the differentiation of preadipose cells into adipose cells [16,17]. As mentioned in section 1, the contribution to the activity of segment 135–146 [1,15] (and therefore of Lys 140 and Lys 145) seems negligible and those of segment 146–191 (containing lysine residues 158 and 168) most likely secondary. It is interesting to point out that in position 64 hGH possesses an arginine residue (which is not modified by our reagents) whereas bGH possesses a lysine residue. This difference might explain the more considerable loss of biological activity observed in the case of guanidinated (or acetimidinated) bGH and suggests that a positive charge at this point of the sequence might play a role in the interaction with the receptors. A closely related molecules hCS, deprived of growth-promoting activity but with almost the same sequence as hGH, possesses at this point a methionine residue instead of a basic residue (Lys or Arg) [19].

It may be concluded from these results that several lysine derived positive charges (including the 64 residue) significantly contribute to the binding of the growth hormone to its rabbit liver receptor. We reported recently that the same kind of chemical modification is accompanied in the case of oCS, another hormone with somatotropic ac-

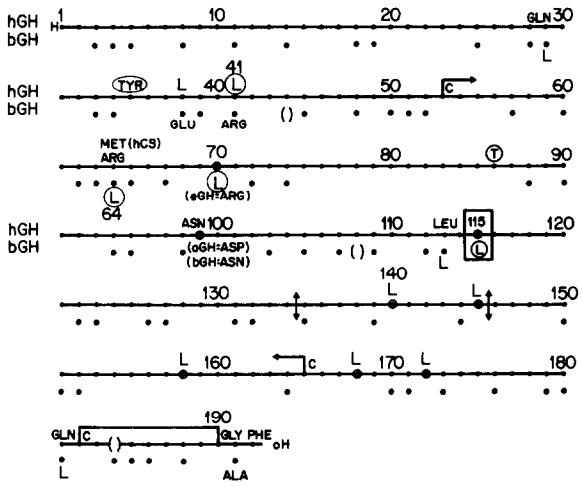

Fig.3. Comparison of primary sequences of human and bovine growth hormones. Amino acid (—●—); GAP ( ); lysine (L); different amino acid in the bGH sequence (not in the line) (●); position occupied by a lysine residue in all growth hormones (⊙); residue is occupied by lysine in growth hormones, prolactins and human chorionic somatomammotropin (hCS) (⊙).

tivity but with unknown amino acid sequence, by a similar or even more drastic loss of biological activity [20]. Thus, residues 41, 64, 70 and 115 are more suitable to play a role not only because a basic residue is always preserved at this position in the GH molecules but also because some of these residues seem to be located in an exposed segment as shown by the reactivity of Tyr 42 [3,13] or by immunological studies with monoclonal antibodies [21].

Residue 115 could also play an important role in relation with the prolactin activity since it is common to all the molecules of the prolactin family.

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