STRUCTURE AND SYNTHESIS OF BIOLOGICALLY ACTIVE PEPTIDES DERIVED FROM PITUITARY GROWTH HORMONE

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Summary. Partial sequence studies on the diabetogenic peptide In-G and on the insulin-like peptide Ac-G from human growth hormone have indicated that the probable sequences of these peptides correspond to residues 164-188 and 1-21 of the growth hormone sequence. These peptides have been prepared by solid phase synthesis and shown to have in vitro biological activities identical with those of the two peptides derived from natural sources.

Previous reports from this laboratory have shown that limited proteolysis of growth hormone from various species yields two biologically active peptides with opposing actions (1-4). One of these (peptide In-G) has biological effects which can account for the diabetogenic action of growth hormone in that it inhibits both triose phosphate dehydrogenases, acetyl CoA carboxylase and, possibly, pyruvate oxidase. These inhibitions result in decreased glycolysis and fatty acid synthesis and increased lipolysis in various tissues. The second peptide (Ac-G) reverses these effects and is hypoglycaemic in normal and diabetic humans (5, 6).

Probable Sequences of the Peptides

Small quantities of the two peptides were prepared from a chromatographically homogeneous sample of human growth hormone, and purified as described previously (2). Partial sequences for each peptide were determined for the amino terminal and carboxyl terminal ends, using a microscale Edman degradation procedure (7, 8) and carboxypeptidase A (9). In both procedures the amino acids were identified and quantitated as their phenylthiohydantoin derivatives, using ^{14}C-phenylisothiocyanate. The amino

terminal residues were also identified as their dansyl derivatives (10).

For Ac-G, the amino terminal sequence was found to be H-Phe-Pro-, while the carboxyl terminal sequence was found to be -Ser-Leu-Leu-Leu-OH. Peptide In-G had the amino terminal sequence H-Arg-Lys-Asp-Met- and the carboxyl terminal sequence -Gly-Phe-OH.

We have been unable to obtain amino acid compositions for the two peptides because only small amounts of material were available. However, it is clear from the absorption spectra of both peptides that neither trytophan nor tyrosine is present. The molecular weight of human In-G by approach to equilibrium measurements in the ultracentrifuge is approx. 2500 (11), while the elution behaviour of the peptides on Sephadex G50 suggests that Ac-G has a slightly lower molecular weight than In-G. Comparison of these partial sequences with the sequence of human growth hormone obtained by Li, Dixon and Liu (12) shows that the amino and carboxyl terminal sequences of the peptides are unique, and permits the unequivocal assignment of Ac-G as residues 1-21 and In-G as residues 164-188 of human growth hormone (Fig. 1). These sequences are consistent with the other observations reported.

Synthesis of the Peptides

Solid phase syntheses of the sequences corresponding to Ac-G and In-G were carried out, by the procedures described by Young and Stewart (13), using an automatic solid phase synthesizer and t-BOC amino acids from Schwarz Biosearch. The peptide was cleaved from the resin and protecting groups removed with anhydrous HF (14).

In both syntheses about 700 mg of amino acid residues were incorporated, corresponding to a yield of ca. 80% on the initial resin ester. Poor coupling was observed with several residues, particularly methionine and asparagine, as evidenced by the amino acid composition. Thus the peptide fractions obtained must be considered to be heterogeneous, and to contain only small amounts of the desired peptides.

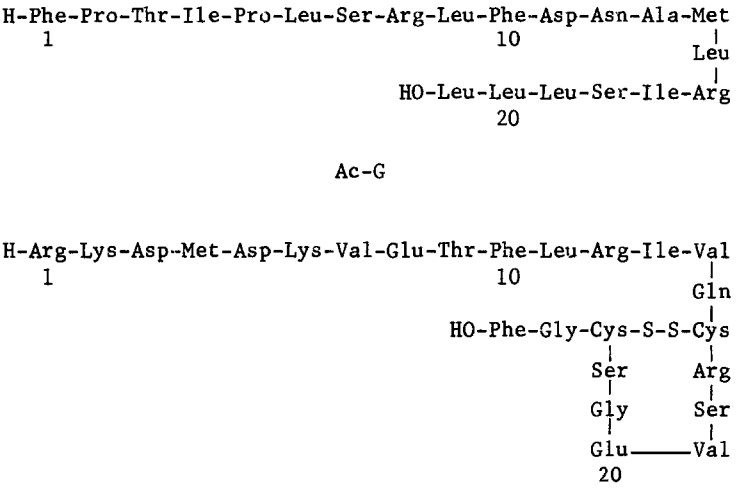

Fig. 1. Probable Structures of Ac-G and In-G from Human Growth Hormone.

Biological Testing of the Synthetic Peptides

The peptide fractions were tested with a number of enzyme systems and tissue preparations which had been studied with the natural materials. The results for natural and synthetic In-G are given in Tables I and II.

TABLE I

The Effect of Natural and Synthetic In-G on the Metabolism of Isolated Tissues

Glucose uptake was measured as described in ref. 3, fatty acid synthesis as described in ref. 1 and lipolysis as described in ref. 4. Thiol and disulphide forms of synthetic In-G correspond to peptide with free thiol groups and after air oxidation, respectively.

	Ovine In-G	Human In-G	Synthetic In-G	
			Thiol	Disulphide
Glucose uptake by soleus muscle	I	I	0	I
Fatty Acid Synthesis by Liver slice	I	I	0	I
Fat pad	I	I	0	I
Lipolysis by fat pad	A	A	0	A

I = Inhibited 0 = Unaffected A = Increased

TABLE II

Effect of Natural and Synthetic In-G on Various Mammalian Enzymes

The conditions for assay of the various enzymes are described in ref. 1.

Enzyme	Ovine In-G	Human In-G	Synthetic In-G	
			Thiol	Disulphide
Dehydrogenases				
Glyceraldehyde-3-phosphate	I	I	0	I
Glycerol-1-phosphate	I	I	0	I
Glucose-6-phosphate	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Hexokinase	0	0	0	0
Aldolase	0	0	0	0
Glutamate oxaloacetate transaminase	0	0	0	0
Acetyl CoA carboxylase	I	I	0	I

I = Inhibited 0 = Unaffected

As prepared, the synthetic In-G has two sulphydryl groups, and it was found that oxidation of these residues was necessary for any activity to be obtained. The appearance of biological activity correlated well with the disappearance of sulphydryl groups. The conditions used for oxidation were those used by White in studies on the oxidation of thiol groups in reduced ribonuclease (15). It may be seen that the oxidised synthetic In-G has the same biological activities and specificity pattern as In-G from growth hormone.

Since natural Ac-G has no effect on the activity of glyceraldehyde-3-phosphate dehydrogenase by itself, and exhibits its activity by reversing the inhibition of the enzyme, a series of tests of natural and synthetic In-G and Ac-G were carried out. The Ac-G as synthesized did not reverse the inhibition of the enzyme. However, it was suspected that the methionine residue at position 14 was present in the synthetic material as its sulphoxide.

Therefore the synthetic Ac-G preparation was treated with sodium sulphite to convert any sulphoxide to methionine (16), and the low molecular weight components (M<1000) were removed by an ultrafiltration washing procedure on a "Diaflo" UM2 membrane (Amicon Corporation, Lexington, Mass.). It may be seen that both natural and reduced synthetic Ac-G reversed the inhibitions produced by either natural or oxidised synthetic In-G.

When the synthetic peptide fractions were subjected to the ion exchange procedure used for the preparation of the natural materials, the biologically active material in the synthetic fractions was found in that part of the elution profile in which the natural materials are obtained.

TABLE III

Effect of Natural and Synthetic Ac-G on the Inhibition of Glyceraldehyde-3-phosphate dehydrogenase by In-G from Various Sources

The activity of glyceraldehyde-3-phosphate dehydrogenase was measured without additions, with In-G or Ac-G, and with In-G plus Ac-G, as described in ref. 3. Active Ac-G is indicated by the reversal of the inhibition produced by In-G. Unless indicated, the synthetic peptide had been reduced with sodium sulphite as described in the text.

Source of In-G	Reversal of In-G Inhibition	
	Ovine Ac-G	Synthetic Ac-G
Ovine	+	-*
Ovine	+	+
Human	+	+
Synthetic (disulphide)	+	+

* Peptide before treatment with sodium sulphite.

Discussion

The results reported here, both on partial sequence studies and on the synthesis and biological activities of the probable structures of Ac-G and In-G, strongly suggest that the diabetogenic activity of growth hormone may be ascribed to residues 164-188 of human growth hormone, and that the insulin-like, hypoglycaemic activity of the hormone is associated with residues 1-21 of the human sequence.

While it is obvious that the synthesized peptides are mixtures of analogous peptides, the fact that the biological activity of the synthetic materials occurs in fractions having the same chromatographic behaviour as In-G and Ac-G prepared from growth hormone is good evidence that the sequences of these peptides are those proposed above.

It is of interest to note that a disulphide bridge is necessary for the biological activity of synthetic In-G. This is presumably intramolecular, since the yields of active material are better if the oxidation of the cysteinyl residues is carried out in dilute rather than more concentrated solutions of the synthetic peptide.

The effect of sodium sulphite on the synthetic Ac-G fraction suggests that a methionine residue is necessary for activity, and that the methionyl sulphoxide form is inactive.

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