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HYPOGLYCAEMIC ACTIVITY OF AN ANALOGUE OF HUMAN GROWTH HORMONE [6-13] INCORPORATING A D-ALA-PRO DIPEPTIDE UNIT

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Abstract: An analogue of human Growth Hormone [6-13] has been prepared incorporating a D-Ala[11]-Pro[12] dipeptide unit to induce a type II' ß-turn conformation. Similar hypoglycaemic activity has been observed to that of the aspartimide-containing analogue Asu[11]-hGH[6-13], indicating conservation of bioactive conformational properties.

The type II' ß-turn has been implicated as an important site for molecular recognition in a number of biologically active peptides and proteins, such as cyclosporin A and gramicidin S.[1] Also, the type II' ß-turn may be induced by aspartimide (α-aminosuccinimide, Asu) formation in peptides and proteins (Table 1) which may alter their biological profile or provide a route to protein repair, degradation or cross linking.[2] As such, this secondary structural motif has been a target for peptide stabilization and mimetic design.[3] Non-α-amino acid substitution has been particularly attractive due to the prospects of increased rigidity and stability. However, such targets carry with them the burden of synthesis, usually in optically pure form. A readily available template for a type II' ß-turn would be particularly useful for the investigation of structure-activity relationships. The use of aspartimide peptides themselves is limited by their hydrolytic instability. Here we describe the use of a D-Ala-Pro motif, which has been shown, by crystal structure and solution spectroscopic analysis, to favour a type II' ß-turn geometry in a synthetic peptide[4]. (Table 1)

TABLE 1 GEOMETRIES OF ß-TURN CONTAINING PEPTIDES

	ϕ_2	ψ_2	ϕ_3	ψ_3
Type II' ß-Turn	60°	-120°	-80°	0°
Boc-Asu-Ala-Gly-OMe	52°	-126°	-96°	12°
Piv-D-Ala-Pro-NH*i*Pr	60°	-140°	-89°	9°

In previous work we have documented the hypoglycaemic activity of an aspartimide-containing analogue of human Growth Hormone, Asu[11]-hGH[6-13] (**1**),[5] and suggested on the basis of supporting crystallographic and solution spectroscopy data, that the induction of a type II' ß-turn may be important for the observed activity.[6] Here we report on the effect of D-Ala-Pro substitution in place of Asu-Asn on hypoglycaemic activity as evaluated by the intravenous insulin tolerance test (IVITT).

The synthesis of the peptide (**2**) was achieved in a straightforward manner using solid phase methods on 4-methylbenzhydrylamine resin. A 25% overall yield was obtained by employing Fmoc-based protection with Ser(tBu) and Arg(Pmc) side chain protection. Protected amino acids were coupled as HOBt esters (2 eq.) in DMF, with 20% piperidine in DMF utilized for deprotection. Preliminary cleavage of the protecting groups with TFA/ethanedithiol/thioanisole/water/phenol (2h), was followed by hard acid cleavage from the resin using TFMSA/TFA/ethanedithiol/thioanisole (2h). It was observed that the use of Fmoc- based side chain protecting groups together with a two-step cleavage procedure, resulted in a product of markedly enhanced purity compared to the standard one-step TFMSA cleavage, particularly with respect to arginine-(Mtr) or -(Mts) deprotection. After isolation and lyophilization the peptide was purified to homogeneity using reversed phase HPLC. Analytical HPLC, FAB-MS and amino acid analysis confirmed the integrity and identity of the peptide [7]

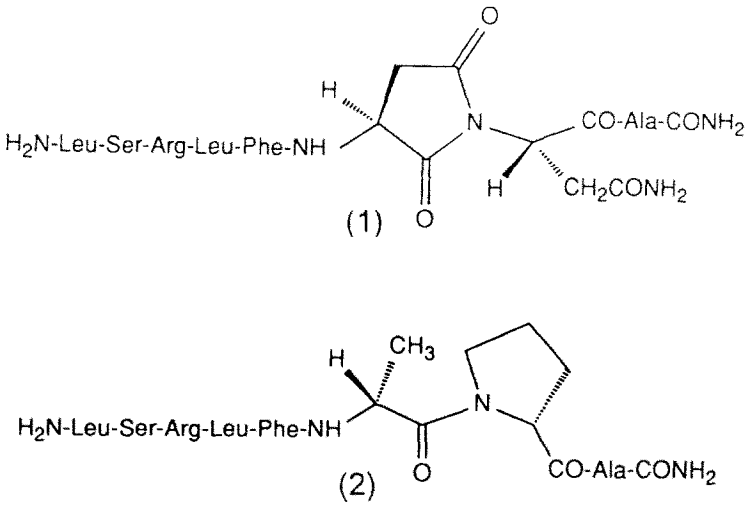

Figure 1 - Structures of Asu[11]-hGH[6-13] (**1**) and D-Ala[11]-Pro[12]-hGH[6-13] (**2**)

The IVITTs[8] were performed to assess biological potency and the bioactivity data compared to the aspartimide-containing peptide (**1**) as a positive control. The results are shown in Figure 2, and clearly document the similarity in activity between the aspartimide-containing peptide (**1**) and the D-Ala-Pro containing peptide (**2**).

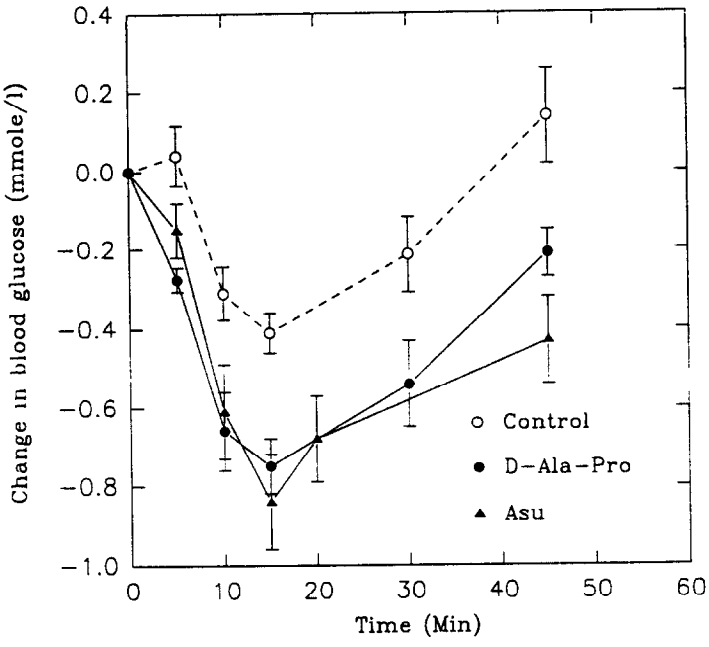

Figure 2 - Comparison of Asu[11]-hGH[6-13] and D-Ala[11]-Pro[12]-hGH[6-13] during IVITTs.

Previously, we have described a γ-lactam containing peptide, which also retains biological activity in the IVITT which we attributed to its conformational analogy to (**1**).[9,10] A range of other analogues showed reduced activity which appears to correlate to the unavailability of a type II' ß-turn conformation.[11] Recent studies, however, have questioned the use of γ-lactam peptides as true type II' ß-turn mimics.[12] The use of non-peptide ß-turn mimics has had some notable successes in the optimization of biologically active peptides and many groups are developing novel templates for the induction or stabilization of ß-turns.[3] For a long time both D-amino acid and proline residues have been recognized as introducing reverse turns in peptides, indeed D-proline has been proposed as a type II' ß-turn mimic, however the two residue combination of these features as in the D-Ala-Pro motif, has until recently been neglected. This and other recent reports suggest that this dipeptide unit may find extensive application in the field of peptide structure-activity studies.[13,14] Furthermore, functionalized versions may provide useful templates where side chain functionality is found to be important. As evident from the results presented here, we have succeeded in preparing an equi-potent analogue of Asu[11]-hGH[6-13]. Such analogues will contribute to the development of a structure-function activity map of hGH[6-13]-related hypoglycaemic peptides, and aid in the development of an orally active non-peptide anti-diabetes drug.

Acknowledgements

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8. Overnight-fasted male Wistar rats (140-160g body weight) were anesthetized with pentobarbital at a dose of 50mg/kg body weight. A blood sample for basal level of glucose was taken, immediately followed by an intravenous injection of saline (control, n=6) or peptide (test, 3mg/kg in saline, n=8). Another blood glucose level was taken after 10 minutes, and the test was then commenced by intravenous administration of insulin (0.10 U/kg body weight). Blood samples were taken at predetermined interval after injection of insulin.
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