

Solid-phase peptide synthesis of human(Nle-27)-oxyntomodulin

Preliminary evaluation of its biological activities

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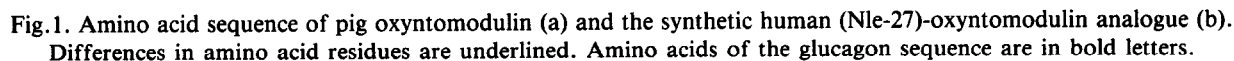
Oxyntomodulin is a peptide isolated from porcine intestine which consists of the whole glucagon sequence extended at its C-terminal part by a basic octapeptide. The analogue (Nle-27)-oxyntomodulin of the human sequence has been synthesized by solid-phase peptide synthesis, purified by HPLC and identified. Its biological activities are the same as those of the natural hormone.

Gastric acid secretion Oxyntomodulin Solid-phase peptide synthesis

1. INTRODUCTION

Oxyntomodulin, a peptide of 37 amino acid residues, has been isolated from porcine jejuno-ileum [1]. This peptide consists of the whole glucagon molecule (residues 1–29) extended at its C-terminal end by an octapeptide [2] of sequence Lys-Arg-Asn-Lys-Asn-Asn-Ile-Ala (fig.1). Recently, the amino acid sequence of human oxyntomodulin has been determined from the human preproglucagon gene [3]. It only differs from the pig sequence by a single replacement in the C-terminal octapeptide, an arginine replacing Lys-33. Glucagon and oxyntomodulin showed different biological activities: on cyclic AMP accumulation in rat liver plasma membranes oxyntomodulin was $\approx 10\%$ as potent as glucagon, but with the same efficacy [2]. In contrast, oxyntomodulin was 20-times more potent than glucagon in stimulating cyclic AMP accumulation in acid-secreting oxyntic glands isolated from the rat gastric mucosa [4]. Similarly, oxyntomodulin was 10–20-times more potent than glucagon in inhibiting pentagastrin-induced acid secretion in the anaesthetized rat [5].

These original characteristics of the molecule are related to the presence of an oxyntomodulin-specific binding site in the rat oxyntic glands [6]. The C-terminal octapeptide of oxyntomodulin which differentiates oxyntomodulin from glucagon, of either the pig or human sequence, was shown to inhibit pentagastrin-induced acid secretion in anaesthetized and conscious rats with the same efficacy as oxyntomodulin but with a lower potency (150-fold) [7–9]. The affinity of oxyntomodulin for antibodies directed against the C-terminal sequence of glucagon was $\approx 1\%$ that of glucagon [2]. The analogue (Nle-27)-oxyntomodulin of the human (and rat) sequence, in which the Met-27 of the natural hormone has been replaced by a norleucine in order to overcome the problems encountered with methionine, was produced by solid-phase synthesis. The replacement of methionine by norleucine has been shown in many cases not to influence the biological activities of the analogues [10–12].



The crude peptide (fig.2) was purified by HPLC, on a μ Bondapak C₁₈ column using a gradient of 0.1% TFA in water/acetonitrile containing 0.05%

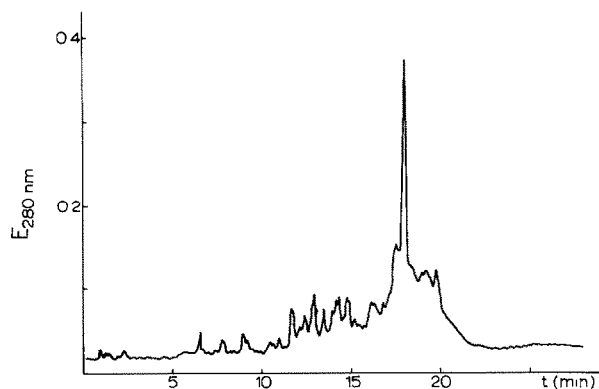


Fig.2. HPLC profile of crude (Nle-27)-oxyntomodulin. μ Bondapak C₁₈ column (10 μ m); gradient, 0–50% solvent B in 50 min (solvent A: water/trifluoroacetic acid, 0.1%; solvent B: acetonitrile/ethyl acetate, 0.05%); flow rate, 1.5 ml/min; detection, UV (280 nm).

ethyl acetate [1]. The apparatus consisted of two solvent delivery systems (model 6000 A) controlled by a solvent programmer (model 720), a U6K injector and two UV detectors (model 441) operated at 280 and 214 nm, respectively. These instruments were from Waters (France). UV spectra (200–300 nm) were directly obtained from the chromatographic peaks using an HP 8450 diode array spectrophotometer (Hewlett-Packard, USA). The purity of (Nle-27)-oxyntomodulin was checked by HPLC on a μ Bondapak CN column and a μ Bondapak C₁₈ column (fig.3).

2.3. Identification of the peptide

Amino acid analysis was performed after acid hydrolysis of the synthetic purified material (6 N HCl, 18 h, 108°C), in the presence of phenol (0.1%) and 2-mercaptoethanol (0.05%) [20], according to Fleury and Ashley [21] and gave the expected values for each amino acid residue: Asp 6.9 (7), Glu 3.3 (3), Ser 3.1 (4), His 0.8 (1), Gly 1 (1), Thr 3 (3), Arg 2.6 (3), Ala 1.6 (2), Tyr 1.7 (2), Val 0.9 (1), Phe 2.1 (2), Ile 1.1 (1), Leu 2.8 (3), Lys 3.2 (3).

The synthetic material was compared by HPLC with natural pig oxyntomodulin on a μ Bondapak C₁₈ and a μ Bondapak CN column, using the conditions of purification. Both showed almost the same retention time.

The UV spectra of the synthetic (Nle-27)-

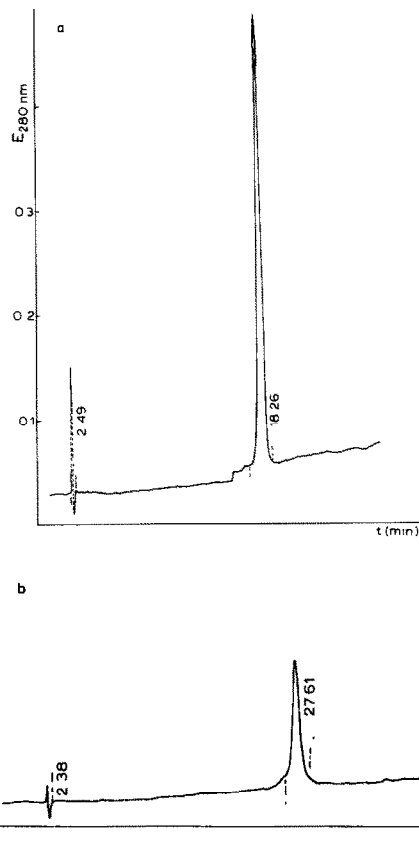


Fig.3. (a) HPLC profile of pure (Nle-27)-oxyntomodulin. μ Bondapak C₁₈ column (10 μ m; Waters); gradient, 0–50% solvent B in 50 min (solvent A: water/trifluoroacetic acid, 0.1%; solvent B: acetonitrile/ethyl acetate, 0.05%); flow rate, 1.5 ml/min; detection, UV (280 nm). (b) HPLC profile of pure (Nle-27)-oxyntomodulin. μ Bondapak CN column (Waters); gradient, 10–40% solvent B in 20 min (solvent A: water/trifluoroacetic acid, 0.1%; solvent B: acetonitrile/ethyl acetate, 0.05%); flow rate, 1.5 ml/min; detection, UV (280 nm).

oxyntomodulin and the natural compound were identical. Only a small difference could be seen between 210 and 230 nm which may be due to the influence of the norleucine and arginine residues of the synthetic material that respectively replace methionine and lysine.

Pure (Nle-27)-oxyntomodulin and glucagon were hydrolyzed with the enzyme *Armilaria mellea* neutral proteinase, which resulted in a complete cleavage at the amino group of the lysine

residues [17]. Both peptides resulted in different fragments which were identified by HPLC and their UV spectra. Glucagon led to peptides 1–11 and 12–29; (Nle-27)-oxyntomodulin led to peptides 1–11 (identical to the 1–11 fragment obtained from glucagon), 12–29 and 30–37. The peptide 30–37 was similar to the octapeptide Lys-Arg-Asn-Arg-Asn-Asn-Ile-Ala formed by solid-phase synthesis and by the conventional technique in solution in our laboratory. Peptides 12–29 produced from the hydrolysis of glucagon and of the synthetic material were slightly different, owing to the difference in the Nle/Met residues.

3. BIOLOGICAL ACTIVITIES

The synthetic peptide showed the same biological activity as the natural hormone. On a molar basis, natural pig oxyntomodulin and the human (Nle-27)-oxyntomodulin analogue possess the same affinity as pancreatic glucagon (G29) for an anti-glucagon antibody prepared in our laboratory [2] (fig.4). With respect to the inhibition of binding of ^{125}I -glucagon to rat liver plasma membranes as described in [1], both peptides

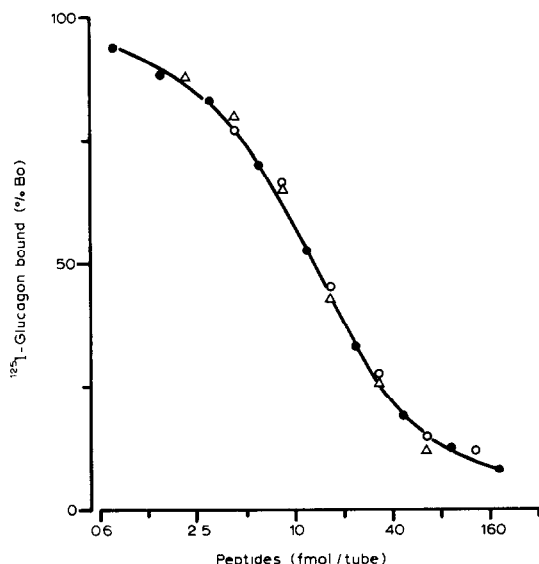


Fig.4. Effects of glucagon (G37) (●), synthetic (Nle-27)-oxyntomodulin analogue (○) and natural pig oxyntomodulin (g37) (Δ) on the binding of ^{125}I -glucagon to an anti-glucagon antibody (666-6) produced in our laboratory.

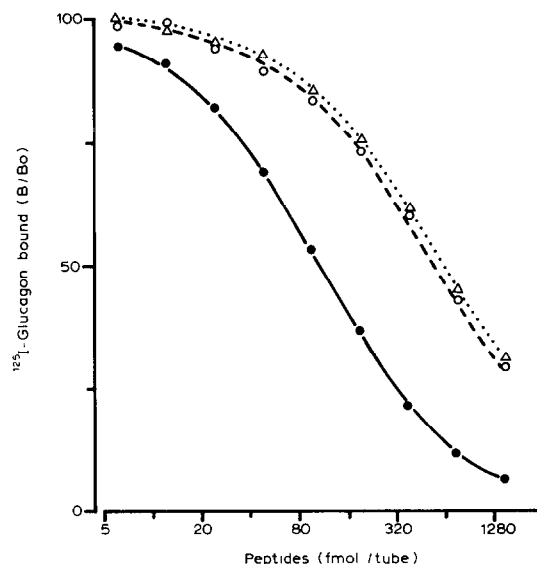


Fig.5. Effects of glucagon (G29) (●), synthetic (Nle-27)-oxyntomodulin analogue (○) and pig oxyntomodulin (Δ) on the binding of ^{125}I -glucagon to rat liver plasma membranes.

behave similarly (fig.5). On the inhibition of pentagastrin-stimulated acid secretion in the conscious rat [9], both the synthetic and the natural material gave the same extent of inhibition (100 pmol of each peptide gave approx. 50% inhibition).

4. CONCLUSION

The synthesis of the (Nle-27)-oxyntomodulin analogue of the human sequence was performed by solid-phase peptide synthesis. The synthetic material was purified, characterized and its biological activities evaluated and compared to those of the natural peptide. As observed in many other examples, this synthesis confirms that the replacement of methionine by norleucine does not affect the biological activity of the hormone, but increases its stability particularly towards oxidation. However, from these preliminary results, apart from some minor differences, it seems that the substitution of Lys-33 (from the pig sequence) by arginine (human or rat sequence) does not have any influence on the biological responses. This synthesis, which allowed us to obtain significant amounts of the (Nle-27)-oxyntomodulin analogue,

will permit us to investigate in further detail the biological activities and probable physiological role of this molecule.

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