branes, was measured by the method of Abrams [8]. Endopeptidase activity was measured by incubating 50 µl of each fraction with 450 µl of peptide-coumarin derivative, each at 0.111 mM in 50 mM-Tris-HCI, pH 7.5. The release of free 7-amino-4-methylcoumarin was quantified fluorimetrically using excitation and emission wavelengths of 370 nm and 440 nm, respectively.

The results obtained for S. cremoris HP are presented in Table 1, and similar results were obtained with S. cremoris AM2. Glucosamine was highest in cell walls of the cellular fractions assayed. The highest levels of magnesium ATPase were found in the cell membrane fraction, whereas the highest levels of lactate dehydrogenase were found in cytoplasm. The majority of the endopeptidase activity as measured by each of the peptide-coumarin derivatives employed was observed in the cytoplasmic fractions with significant levels of activity in the cell membrane fractions. Little or no activity against the peptide-coumarin derivatives could be found in the extracellular fluid, wash or cell wall fraction. It is of note that the previously mentioned proteinase activity of cell walls could not be detected by these substrates. Our results indicate that the endopeptidase activities observed using peptide-coumarin derivatives as substrates, being predominantly located in the cytoplasm, are likely to be significant in cheese ripening only on the lysis of the cell.

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Proteolysis of the zona pellucida of mouse ova

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During the process of fertilization of the ovum, the acrosome of the spermatozoa engages in an exocytotic event and releases hydrolytic enzymes [1]. The acrosome originates during spermiogenesis via vesicle formation by the Golgi apparatus and is considered to be a modified lysosome. The acrosome contains several hydrolytic enzymes and those that have been investigated were found to differ both biochemically and immunologically from their lysosomal counterparts [2]. Fertilization requires that the spermatozoa penetrate the cumulus mass, the zona pellucida and the vitelline membrane. The cumulus cells are dispersed by the action of hyaluronidase and penetration of the zona pellucida is reported [3] to be due to a trypsin-like enzyme named acrosin: The properties of this enzyme have not been fully described. The zona pellucida was not affected by exposure to collagenase, lysozyme or phosphodiesterase, but was dissolved rapidly by Pronase, a commercial preparation of extracellular enzymes secreted by Streptomyces griseus [4]. In the work presented here we describe a partial purification of the zona pellucida lytic activity from Pronase.

Pronase (Boehringer Corp., Mannheim, F.R.G.) was fractionated by cation-exchange chromatography on carboxymethyl cellulose (CM-52, Whatman, Maidstone, Kent, U.K.) according to the method of Narahashi [5]. Pronase (0.5 g) was dialysed against 0.01 M-sodium acetate buffer, pH 5.2, containing 5 mM-calcium chloride, and applied to a column (21 cm x 1.5 cm) of CM-52 cellulose previously equilibrated with the same buffer. The column was washed with the starting buffer until all non-absorbed proteins were eluted and the column was then irrigated with a gradient (0–0.25 M) of sodium chloride. Fractions (5 ml) were collected and their absorbances at 280 nm, together with their proteolytic activities, were determined. Total proteolytic activity was determined using haemoglobin as substrate and measuring,
using the Folin-phenol reagent, the release of peptides soluble in 5% (w/v) trichloroacetic acid [6]. Trypsin activity was determined using benzoyl-L-arginine ethyl ester as substrate [7] and chymotrypsin by the hydrolysis of succinyl phenyl-4-nitroanilide. The fractionation of pronase was also monitored both by (1) electrophoretic analysis of the fractions on polyacrylamide gels in the presence of urea [8] and staining with Coomassie Blue; and (2) by enzymography using polyacrylamide gels containing gelatin [9]. To measure the zona pellucida lytic activity, the pronase fractions were dialysed extensively against phosphate-buffered saline containing 136 mm-NaCl, 2 mm-KCl, 6.5 mm-NaHPO$_4$ and 1.5 mm-KH$_2$PO$_4$ (pH 7.4) and incubated with freshly ovulated mouse ova. The ova were collected from quail embryos which had been induced to superovulate by injection of 5 i.u. of follicle-stimulating hormone followed 48 h later by 5 i.u. of choricionic gonadotrophin.

The zona pellucida activity of Pronase was abolished by the presence of any one of the following: aprotinin, phenylmethylsulphonyl fluoride, soybean trypsin inhibitor (Sigma Chemical Corp., Poole, Dorset, U.K.) and N-p-tosyl-L-lysine chloro methyl ketone (Boehringer Corp., Mannheim, F.R.G.) at a final concentration of 10$^{-5}$ M. Zona lysis activity was stable at neutral pH but unstable at low pH. Zona lytic activity survived heating to 50°C, at pH 7.4, for 60 min, and its activity was not affected by added EDTA or N-ethylmaleimide.

Chromatography on CM-cellulose separated the trypsin and chymotrypsin activities from each other and from other proteolytic activities. Only the chymotrypsin-containing fractions caused lysis of the zona pellucida.

Polyacrylamide-gel electrophoresis in the presence of urea showed Pronase to contain a complex array of intermediate and low-molecular weight proteases. The zona lytic activity from CM-cellulose was heterogeneous with respect to protein content but when assayed on enzymography gels showed a single enzymic activity which migrated with an approximate molecular size of M, 20,000.


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1H-nuclear magnetic resonance conformational studies on synthetic analogues of gastrin-releasing peptide

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Introduction

Gastrin-releasing peptide (GRP) and its amphibian counterpart bombesin (BN) exhibit a wide range of biological activities in mammals, but they have attracted particular attention since the discovery that they can act as potent autocrine growth factors in human small-cell lung carcinoma [1].

There has been considerable interest in the design of competitive GRP/BN antagonists as possible antimitotic agents and to understand the structure–activity relationships of this peptide family. Full bombesin activity is found in C-terminal peptides of seven to nine residues [2]. Hence, we have begun a programme to examine the role of specific regions of GRP in the binding of BN and to understand the structure-activity relationships of this peptide family. Full bombesin activity is found in C-terminal peptides of seven to nine residues [2]. Hence, we have begun a programme to examine the role of specific regions of GRP in the binding of BN and to understand the structure-activity relationships of this peptide family.

A series of peptides have been designed and synthesized incorporating the conformationally constraining residue 1-aminoacyclopropane-1-carboxylic acid (Ac,c) as well as D-amino acid substitutions in an attempt to induce the desired β-bend.

We report here the results of 1H-n.m.r. studies on one of these peptides (analogue 1), D-, Glu-Trp-Ala-Val-Ac,c-D-Phe-Leu-Met-NH$_2$. This analogue has been shown to possess mitogenic activity equal to that of GRP (18-27) in the human breast cancer cell line ZR-75-1 [6].

Methods

All peptides were synthesized by solid-phase methodology using fluoren-9-ylmethoxycarbonyl-protected amino acids and Pepsyn KB resin (Cambridge Research Biochemicals Ltd., Cambridge, U.K.) [7]. Purification involved preparative h.p.l.c. on a Waters Delta-Prep system using a C-18 reverse-phase column. Sequences were confirmed by amino acid analysis and fast atom bombardment mass spectrometry.

One- and two-dimensional correlation (COSY) and one-dimensional nuclear Overhauser enhancement (NOE) difference spectra were recorded at 278K on a Bruker 360 MHz spectrometer. Complete assignment of the backbone and many side-chain protons was achieved using the COSY and NOE spectra.

Results and discussion

Conformational studies on GRP/BN show that these peptides do not adopt a predominant conformation in solution...