

In the scallop *Pecten maximus*, the enzyme α -amylase is present in the digestive complex of the digestive gland where intra and extracellular processes occur. Its purification from the digestive gland has been performed using an affinity chromatography on modified starch (Minamiura et al, 1975). Two isoforms were present and the molecular weight was estimated at 60 000 on SDS gel electrophoresis.

In order to have information on its structure and regulation, a digestive gland cDNA library, constructed in lambda phage Zap II (Stratagene) was screened with a crustacean α -amylase cDNA probe (Van Wormhoudt and Sellos, 1996). Only 0.02% of the clones were positive and the longest clone, having a size of 1 700 bp and identical to that of the mRNA, was fully sequenced. It contains the complete cDNA coding frame for one of the amylase isoforms of *P maximus*. The deduced protein sequence is 508 amino-acids long, including the initial methionine, and corresponds to an 18 amino-acid highly hydrophobic signal peptide and a mature enzyme of 489 residues. The calculated molecular weight corresponds to 54 500 Da and the pHi to 6.76.

The consensus regions that characterize the α -amylase catalytic domains and the three amino-acids belonging to the active site are found in *Pecten*, confirming the high level of similarity with other amylases. A same amino-acid identity was determined with arthropod (57% homology with *Penaesus*, a marine crustacean, 55% homology with *Drosophila*) and vertebrate (60% homology with human) amylase sequences. The cDNA will be used to study mRNA expression in relation to nutritional and endocrine regulation.

Data obtained by both methods are in agreement. N-terminus serine, deduced from the nucleic sequence may be acetylated, preventing Edman degradation and direct knowledge of amino-acid sequence and explaining the lower molecular weight of

the molecule estimated from the nucleotide sequence.

References: Minamiura N, Kimura Y, Tsujino K, Yamamoto T (1975) *J Biochem* 77, 163-169; Van Wormhoudt A, Sellos D (1996) *J Mol Evol*.

Digestive effects of algal dietary fibres in humans. N Bentoumou, C Cherbut (*Centre de recherche en nutrition humaine, Inra, BP 1627, 44316 Nantes cedex 03, France*).

Dietary seaweeds are rich in fibre which could affect stool output and intestinal transit time in humans. We compared the digestive effects of three fibres isolated from seaweeds: xylans from *Palmaria palmata* (PP, 10 g/day), carragenans from *Eucheuma cottonii* (EC, 10 g/day), and alginates from *Laminaria digitata* (LD, 6 g/day) to those of cellulose (Cel, 10 or 6 g/day) in 18 healthy subjects. The study was designed in two experimental periods (21 days), separated by 15-day wash-out. During each period, the subjects consumed a basal diet, containing less than 10 g/day of dietary fibre, added with cellulose or algal fibres in a balanced randomised order. All the stools excreted during each period were collected then analysed (dry matter, water content, short chain fatty acids: SCFA). The orofaecal transit time (OFTT) was measured using radio-opaque markers. The data (mean \pm SE) were compared by a Student's *t* test for paired data.

EC doubled faecal wet weight (EC vs Cel: 293.7 \pm 24.6 g/day vs 152.1 \pm 19.1 g/day, $P = 0.0002$) by increasing water output (277.9 \pm 21.9 g/day vs 104.7 \pm 13.0 g/day, $P = 0.0006$). This effect is likely to be related to the high water-binding capacity of EC which resisted fermentation as suggested by the high value of stool pH (7.23 \pm 0.11 vs 6.47 \pm 0.15, $P < 0.05$) and the low faecal SCFA output rate (134.8 \pm 30.2 mmol/day vs 164.5 \pm 26.6 mmol/day, $P <$

0.05). PP and LD had no laxative effect. Nevertheless, these two fibres modified stool composition by increasing water content: $78.4 \pm 2.1\%$ (PP) and $76.8 \pm 1.9\%$ (LD) vs $68.5 \pm 2.6\%$ (Cel), $P < 0.05$. Neither EC nor PP changed OFTT, whereas LD increased it (96.6 ± 19.8 h vs 64.9 ± 15.3 h, $P = 0.016$), which could be due to its high viscosity.

In conclusion, the fibres isolated from dietary seaweeds exert digestive effects which depend on their physico-chemical and fermentative properties. Among the tested fibres, EC appears as an efficient laxative well accepted by the subjects.

Colo-ileal refluxes modulate terminal ileum motility in pig. G Cuche, CH Malbert (*Inra, station de recherches porcines, 35590 Saint Gilles, France*).

The terminal ileum exhibits typical motor patterns, Discrete Clustered Contractions (DCC) and Prolonged Propagated Contractions (PPC), induced by ileal infusion of caeco-colonic compounds [Kruis et al (1985), *Am J Physiol* 249, G264-270; Kamath et al (1987), *Am J Physiol* 253, G427-433]. The aim of this study was to evaluate: *i*) the possible reflux of colonic contents in the terminal ileum; *ii*) the relationship between these refluxes and ileal motor events.

In four conscious pigs, the motility of the terminal ileum was evaluated using three strain gauges sutured 15, 10 and 5 cm proximal to the ileocecal sphincter (ICS). The ileal pH, used as an indicator of coloileal refluxes [Roger et al (1990), *J Gastrointest Motil* 2, 224-229], was continuously measured 5 and 10 cm proximal to the ICS.

During interdigestive and postprandial periods, frequent pH dips were recorded. 82% of these dips were detected successively by the two pH probes. Their frequency (8 ± 0.5 vs 6 ± 0.4 h⁻¹; interdigestive vs postprandial, $P < 0.05$), duration ($262 \pm$

12.7 vs 356 ± 27.0 sec, $P < 0.05$), but not amplitude (0.6 ± 0.02 vs 0.6 ± 0.03 pH, NS) were modified by feeding. The frequency of DCC increased during pH dips (0.6 ± 0.18 vs 1.6 ± 0.15 contraction.min⁻¹, $P < 0.05$). The onsets of 46% of the dips were preceded by more frequent retrograde contractions. On the contrary, while pH went back to basal level, contractions were mainly aborally propagated. About half of PPC occurred 6 ± 0.9 sec before the end of the pH decrease. The amplitude (0.4 ± 0.06 pH) and duration (118 ± 26.0 sec) of pH dips associated with PPC were reduced compared to those not temporally related to pH dips ($P < 0.05$).

In conclusion, the last 10 cm of the ileum are periodically invaded by caecocolonic fluids. These acidic refluxes are temporally related to ileal motility.

Systemic and mucosal immune responses after oral administration of bovine lactoferrin in mice. H Debbabi, M Dubarry, M Rautureau, D Tomé (*Unité Inra de nutrition humaine et de physiologie intestinale, INA-PG, 16, rue Claude Bernard, 75005 Paris, France*).

Lactoferrin (Lf) is an ironbinding glycoprotein presents in milk, in different external secretions, and in specific secondary granules of neutrophils. Bovine Lf has been shown to have bacteriostatic and immune functions. As Lf acts also as an oral antigen, we have studied the systemic and mucosal immune responses after oral administration of bovine Lf in mice.

Three groups of mice were force-fed during 4 weeks with either: 1 mg/day (Lf1), 20 mg/day (Lf20) of bovine Lf or water (T) as control. The humoral immune response was determined in mucosal secretions and in sera. IgA and IgG productions and IL 2 and IL-5 secretions by B lymphocytes and T helper lymphocytes respectively were mea-