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Gastric lipase: an extremophilic interfacial enzyme with medical applications

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Gastric lipase is active and stable in the acidic environment of the stomach, where the gastrointestinal lipolysis of dietary fat is initiated [1]. This lipase can be said to be an extremophilic enzyme because it retains its activity in gastric juice fully at pH 2, in the presence of pepsin and at physiological temperature [2]. In addition, gastric lipase is not inhibited by the bile salts present in the gastrointestinal (GI) tract, whereas pancreatic lipase, the main lipase involved in fat digestion, requires a cofactor, namely colipase, to counteract the inhibitory effects of bile salts on lipase adsorption at lipid-water interfaces [3]. In view of its unique properties, gastric lipase was chosen as a candidate for the treatment of the pancreatic enzyme insufficiency observed in chronic pancreatitis and cystic fibrosis (CF). In these pathologies characterized by exocrine pancreatic secretion deficiency, gastric acid secretion is not neutralized by pancreatic bicarbonate, and the pH of the small intestine contents can reach values as low as 2 – 3 [4]. In replacement enzyme therapy, the use of acid-resistant lipases can be expected to yield more satisfactory results than the porcine pancreatic extracts currently prescribed. In addition, these pancreatic enzyme preparations of animal origin present a potential risk of viral transmission to humans and there is a high pressure for developing novel enzymes produced by genetic engineering.

Several trials for producing gastric lipase in various expression systems have been performed for developing industrial production during the last 20 years. An active recombinant human gastric lipase (HGL) was found to be produced in the yeast *Saccharomyces cerivisae* and in insect cells. Neither system, however, was suitable for industrial production since the lipase either remained stacked to the yeast cell wall [5, 6] or was produced at low levels in insect cells (milligrams per liter of culture [7]). The fact that the enzyme should be produced at a low cost to replace the existing porcine pancreatic extracts led the pharmaceutical laboratories to investigate the production of a recombinant gastric lipase in transgenic plants. Dog gastric lipase (DGL) was selected because it is the lipase showing the highest hydrolytic activity on long-chain triglycerides at low pH levels, with an optimum activity at pH 4 [8]. The cDNA encoding DGL was obtained [9] and used to transform tobacco [10] and maize with the gene-gun technique. A recombinant DGL was produced at high levels in both tobacco leaves and maize seeds (approx. 1 mg rDGL per seed; data communicated by Meristem Therapeutics SA, Clermont-Ferrand, France). This enzyme was given the status of 'orphan medicinal product' by the European Medicines Agency (EMEA) in 2003 for the treatment of cystic fibrosis patients, and clinical

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use of the rDGL purified from maize seeds is currently under investigation.

Beside these industrial and clinical developments, our basic knowledge on the molecular aspects and the physiopathology of gastric lipase has been extended [3, 11–13]. Understanding the optimum activity of gastric lipase at acidic pH values was a major challenge, and a recent study revealed how DGL preferentially adsorbs at the lipid-water interface at low pH [14]. Beside these new insights on the interfacial catalysis performed by gastric lipase, the idea that this enzyme might be a good candidate for replacement enzyme therapy was supported by the finding that its secretion increases naturally in patients with chronic pancreatitis [4].

How gastric lipase – an interfacial enzyme with a Ser-His-Asp catalytic triad – acts optimally at acidic pH

Like those of other lipases, the three-dimensional (3D) structure of gastric lipase is based on an α/β hydrolase fold and its active site, which is covered by an amphiphilic lid domain, comprises a Ser-His-Asp catalytic triad and an oxyanion hole [11, 12]. Most lipases show optimum activity at pH levels above 7, which is consistent with the ionization properties of histine (pKa=6.5) and the fact that this residue is known to play an important role in the charge relay system involving the catalytic triad and the enhancement of the nucleophilic character of the serine residue. It was therefore of great importance to understand the unusual optimum activity of gastric lipase occurring at acidic pH values, and many efforts have been made to perform structural studies for this purpose. The 3D structures of human (HGL) and dog (DGL) gastric lipases were not found, however, to show any specific features at the level of the catalytic triad, which was superimposable on all the catalytic triads observed in other lipases and esterases so far [11, 12]. One might expect the catalytic histidine residue His353 to show a strong local pKa decrease to explain the acidic pH optimum of gastric lipase. However, no additional charged residues were observed within a 10-Å sphere centered on the active site Ser153 Oγ atom. It was therefore impossible to postulate any mechanism for a local change in the histidine pKa from the gastric lipase 3D structure.

Recently, it was shown however that gastric lipase acts in solution on a partly soluble substrate (vinyl butyrate), with an optimum activity above pH 7, which suggests that gastric lipase is able to hydrolyze ester bonds via the classical mechanism of serine hydrolases [14]. The optimum activity of gastric lipase is shifted toward lower pH values, however, when the vinyl butyrate concentration is greater than the solubility limit. Since the lipase adsorption at the lipid-water interface must occur before the insoluble substrate is hydrolzed (Fig. 1), these experiments suggested that the lipase adsorption step was also pH dependent. Experiments performed with long-chain triglyceride emulsions confirmed that gastric lipase binds optimally to the oil-water interface at low pH values [14]. To study the effects of pH on the adsorption step independently from substrate hydrolysis, gastric lipase adsorption on solid hydrophobic surfaces was monitored by total internal reflection fluorescence (TIRF), as well as using a quartz crystal microbalance (QCM). Both techniques showed a pH-dependent reversible gastric lipase adsorption process, which was optimum at acidic pH values. These results indicated that the optimum activity of gastric lipase at acidic pH is only 'apparent' and results from the fact that lipase adsorption at lipid-water interfaces is the pH-dependent limiting step in the overall process of insoluble substrate hydrolysis (Fig. 1).

Contrary to what is observed with 'classical' enzymes acting on soluble substrates, the kinetic properties and substrate specificity of interfacial enzymes can result from both the adsorption of the enzyme at the lipid-water interface and the interactions occurring between the substrate and the active site. Studies on gastric lipase have shown how these essential reaction steps can be influenced by pH variations, and this specific kinetic feature of interfacial enzymology should be taken into account when studying any soluble enzyme acting on an insoluble substrate.

Gastric lipase secretion is drastically increased in patients with severe chronic pancreatitis

Quantitatively, human pancreatic lipase (HPL) is the main enzyme involved in the digestion of dietary fat, but has now been established that HGL can release from 10 to 25 % of dietary triglyceride acyl chains in the stomach and continue its action in the small intestine together with HPL [1, 3, 15]. In cases of pancreatic exocrine insufficiency, compensation for the loss of HPL by HGL was suggested by several studies. The lipolytic activity of gastric contents was sometimes found to be higher in CF patients than in control subjects during test meals [16]. It has been shown that the secretory output of HGL under pentagastrin stimulation was significantly increased in the late stage of alcoholic chronic pancreatitis (CP) [17]. We recently showed that HGL outputs are clearly increased (three- to fourfold vs. controls; Fig. 2) in the late stage of CP during test meals [4].

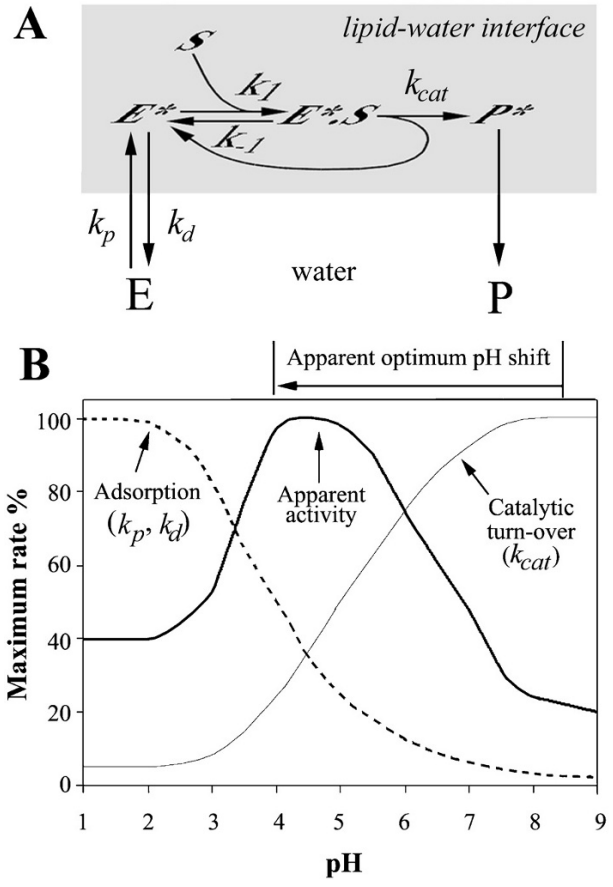

Figure 1. Action of gastric lipase on an insoluble triglyceride substrate. (*A*) Kinetic model showing the enzyme adsorption step (E-E*) at the lipid-water interface, followed by the formation of a Michaelis-Menten enzyme-substrate complex at the interface and the release of the reaction product [23]. (*B*) Simulation of gastric lipase apparent activity taking into account pH-dependent enzyme adsorption and catalytic turn-over.

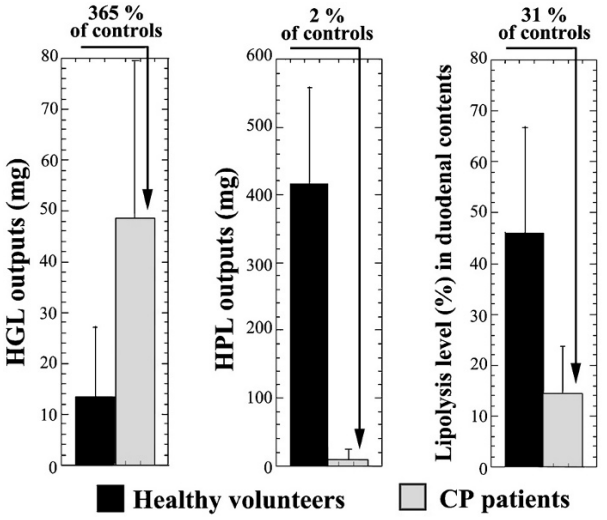

Figure 2. Gastric and pancreatic lipase secretions and contribution to gastrointestinal lipolysis in healthy subjects and patients with severe chronic pancreatitis (Adapted from Carrière et al. [4]).

The level of lipolysis measured in the duodenal contents collected for 3 h was 31.4 % of that measured in healthy volunteers (Fig. 2). Since pancreatic lipase secretion was almost completely abolished in severe CP patients (2.2 % of control subjects; Fig. 2), these results indicate that HGL alone is able to convert one third of the ingested fat into absorbable free fatty acids and monoglycerides, and they are in line with several previous findings made on CF and CP patients who were still able to absorb large amounts of dietary lipids. In CF patients, 20–80 % of the ingested triglycerides remain absorbed [18–21]. In the very rare existing cases of total congenital pancreatic lipase deficiency, 50 % of the ingested triglycerides can be absorbed [22].

Although gastric lipase alone cannot normalize overall lipolytic activity, it was shown on a quantitative basis that gastric lipase can partly compensate for the loss of pancreatic lipase in cases of severe exocrine pancreatic insufficiency [4]. This finding provides valuable support for the clinical development of gastric lipase in pancreatic enzyme replacement therapy.

One might expect that the substantial advances made in the characterization of gastric lipase should trigger the clinical research in this area, but the current debate on the use of genetically modified organisms seriously impairs the ongoing projects. In France, for example, several fields in which transgenic maize was used to produced rDGL were destroyed by active groups fighting against the development of genetically modified organisms (GMO). The fact that these transgenic plants are used to produce recombinant proteins for medical application appears not sufficient to convince public opinion that the benefit of such novel drugs can balance the potential risks of spreading GMOs in the nature. As a consequence, the costs of development, and therefore the cost of the final product, have increased tremendously due to the various safety procedures implemented along the process of recombinant protein production. Finding financial support for such projects is becoming increasingly difficult and it is uncertain whether 'Molecular Pharming' will achieve the development expected some years ago. One can hope, however, that the new technologies already implemented for producing transgenic plants will change the mind of public opinion about the risks associated with GMOs. The transgenic maize currently grown in France and other countries for producing rDGL does not, unlike the early transgenic plants released in nature, contain any gene for resistance to antibiotics. Moreover, the genetically modified crops are male sterile and cannot give rise to new generations of transgenic maize.

Fertilization and production of maize seeds is ensured by growing wild-type maize together with transgenic maize in the same fields (personnal communications from Dr., D. Mison, Meristem Therapeutics, Clermont-Ferrand, France). The future of transgenic plant use will probably depend as much on communication as on scientific breakthrough.

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