

Amadori Products – Substrates and Inhibitors for Intestinal Brush Border Glycosidases

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Abstract: The effect of oligosaccharide-derived Amadori products on intestinal disaccharidases was investigated to answer the question, whether these products, ingested in relevant amounts with the daily diet, are degraded by carbohydrate-digesting enzymes and in addition interfere with the digestion of other carbohydrates. Using Caco-2 cells, which served as model for the intestinal brush border, the hydrolysis of peptide-bound Amadori products, prepared from N α -hippuryllysine and maltose or maltotriose, respectively, as well as of the oligosaccharides was studied. The Caco-2 cells hydrolysed the Amadori products to yield N α -hippuryl-fructosyl-lysine and D-glucose. The inhibition of Caco-2 sucrase-isomaltase by Amadori products was examined with a microtitre plate assay with *p*-nitrophenyl- α -D-glucopyranoside as substrate. IC₅₀ values were in the millimolar range, indicating that Amadori products do not act as strong inhibitors for intestinal glycosidases. The hydrolysis of the natural substrate maltose, on the other hand, was barely affected.

Keywords: Caco-2 cells; oligosaccharide; Amadori compounds; α -glucosidase inhibition

INTRODUCTION

Amadori products (APs) are the first and quantitatively most important products of the Maillard reaction. Up to 70% of the initial lysine content in protein may be present as APs (HENLE 2003). Besides glucose, the oligosaccharides lactose, maltose and maltotriose can be expected to be important precursors for APs in dairy products, bread or beer. The fate of the AP N ϵ -fructosyl-lysine (FL) in the gastro-intestinal tract has been investigated for years (ERBERSDOBLER & FAIST 2001). Only limited information, however, is available concerning the handling of oligosaccharide-derived APs by intestinal glycosidases (SCHUMACHER & KROH 1994), which comprise soluble α -amylase and the three brush border membrane bound complexes sucrase-isomaltase (SI), maltase-glucoamylase (MGAM) and lactase-phlorizin-hydrolase (LPH).

A widely used model for the human small intestine to study effects of α -glucosidase inhibitors is the human colon cancer cell line Caco-2, due to its ability to express membrane bound SI and LPH, when spontaneously differentiated at

confluency (CHANTRET *et al.* 1994). Using this model, we investigated the inhibitory properties and the hydrolysis of peptide bound oligosaccharide APs.

METHODS

Peptide-bound APs were synthesised from N α -hippuryl-lysine and maltose or maltotriose according to KRAUSE *et al.* (2003). The Caco-2 cells were seeded at 2×10^5 cells per cm² into microtitre plates (96 wells and 24 wells) and cultured with MEM medium (1 g/l glucose) supplied with L-glutamine, 10% FBS, 1% non-essential amino acids and 80 mg/l gentamicin (KNÜTTER *et al.* 2008). The assays were performed with cell cultures 10–14 days post confluency. The α -glucosidase activity was measured with the substrate *p*-nitrophenyl- α -D-glucopyranoside (pNPGlc, 3mM in 100 μ l 50mM MES-buffer, pH 6.0) at 37°C directly on the cell layer in 96-well-plates. By adding 100 μ l 100mM Na₂CO₃, the reaction was stopped after 45 minutes. The absorbance at 405 nm corresponds to

the amount of liberated *p*-nitrophenolate (external calibration), which reflects substrate conversion. Inhibitory properties were determined by adding different concentration of the inhibitor and fitting the activity values to a Boltzmann curve with Origin[®] to result in IC_{50} values, which is the concentration of an inhibitor for 50% inhibition of the enzyme. Investigations on the hydrolysis of oligosaccharide-derived APs and of maltose by the cell layer were performed with 25mM isotone MES-buffer, pH 6.0, at 37°C (KNÜTTER *et al.* 2008). Liberated glucose was measured with a hexokinase-based glucose assay kit, hippuryl-APs with RP-HPLC-UV (KRAUSE *et al.* 2003).

RESULTS

The assay of α -glucosidase activity of Caco-2 cells with pNPGlc showed a maximum velocity of 0.60 ± 0.03 mU/cm², which was linear during the incubation time. When adding inhibitors, the activity was reduced as shown in Table 1. The IC_{50} value of acarbose, a well-known α -glucosidase inhibitor, is in agreement with published data of 0.05mM for porcine small intestinal α -glucosidase (KIM *et al.* 2004). N^α-hippuryl-N^ε-maltulosyl-lysine (HML) inhibited weakly. At equal concentrations of the substrate pNPGlc and HML, the activity towards pNPGlc was reduced to approximately 50%, suggesting an equal binding affinity. N^α-hippuryl-N^ε-maltotriulosyl-lysine (HM3L) was less inhibiting than HML, even though it conserves a maltose moiety within the molecule. Interestingly, the natural substrates of SI, maltose and maltotriose, inhibited the pNPGlc hydrolysis less than HML did. To

Table 1. Inhibitor concentration to reduce pNPGlc-hydrolysing activity of Caco-2 cells to 50% (IC_{50}). pNPGlc 3mM, pH 6.0 (MES-buffer), 45 min incubation. Values given with SD are means of duplicates

Inhibitor	IC_{50} (mM)
N ^α -hippuryl-N ^ε -maltulosyl-lysine	4 ± 1
N ^α -hippuryl-N ^ε -maltotriulosyl-lysine	16
Acarbose	0.06 ± 0.04
Maltose	11
Maltotriose	11
Hippuric acid	32

examine any inhibition caused by the AP-modification itself, hippuric acid (N-benzoylglycine) served as control. It did not exhibit considerable inhibitory properties ($IC_{50} = 32$ mM).

In a second approach, the cells were incubated with HML or HM3L, respectively, as substrates at varying concentrations (0.15 to 0.33mM). The relative amount of the reaction products was quantified with RP-HPLC-UV. As shown in Figure 1, HM3L was hydrolysed by the cells to HML, and this was further degraded to N^α-hippuryl-N^ε-fructosyl-lysine (HFL). At the same time, D-glucose was released, as was confirmed with a glucose assay kit. The maximum enzyme activity was much lower than the one using pNPGlc as substrate (0.03 mU/cm² for HML compared to 0.6 mU/cm² for pNPGlc). HM3L is a slightly better substrate than HML, since the maximum activity was not achieved under assay conditions and therefore is higher than 0.06 mU/cm². The presence of HML during the incubation of cells with maltose barely

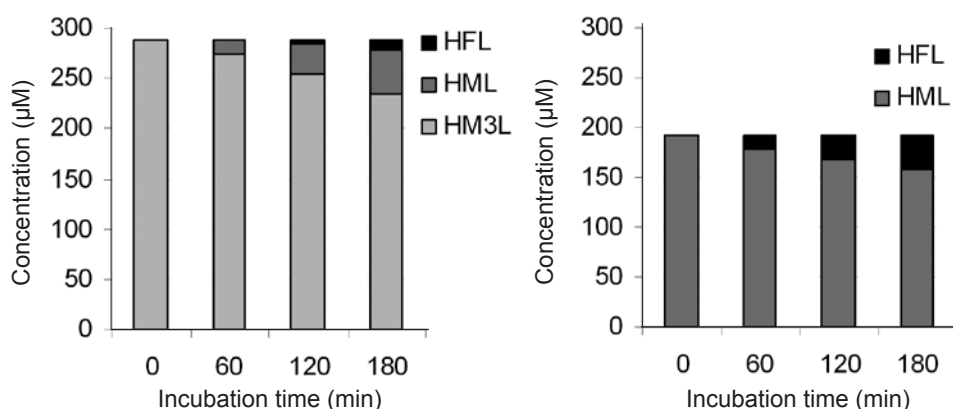


Figure 1. Hydrolysis of Amadori products HM3L and HML by Caco-2 cells in wells with 2 cm² cell layer and 0.4 ml incubation buffer (isotone 25mM MES, pH 6.0, 37°C). (a) HM3L 0.29mM, (b) HML 0.19mM. HFL and glucose (not shown) are the end products

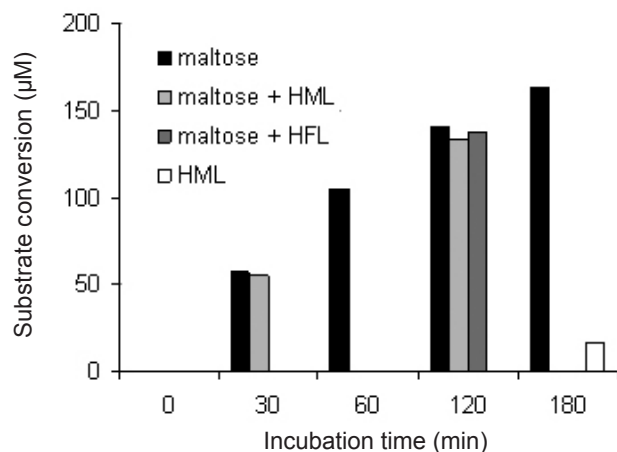


Figure 2. Maltose hydrolysis by Caco-2 cells in presence of HML. 2 cm² cell layer, 0.4 ml buffer (isotone 25mM MES, pH 6.0) with 0.38mM maltose and in some cases 0.38 mM HML or HFL. Substrate conversion calculated from glucose liberation

affected the maltose hydrolysis rate, which was in the range of 0.2 mU/cm² during the start of the incubation (Figure 2). The rate might be underestimated, for it is not yet clear, which amount of glucose is absorbed by the cells and therefore is not recorded.

In conclusion, it could be shown that the peptide-bound APs HML and HM3L are slowly but efficiently hydrolysed by α -glucosidase activity (sucrase-isomaltase) expressed by Caco-2 cells. Therefore, oligosaccharide-derived APs ingested with daily food should be completely hydrolysed to monosaccharide APs during their intestinal passage.

The inhibitory potential of APs on carbohydrate digestion remains unclear. The IC₅₀ values of HML and HM3L using pNPGlc as substrate are in the same order of magnitude as those of the natural substrates maltose and maltotriose, but HML does hardly inhibit maltose hydrolysis directly. This may be due to different hydrolysis kinetics of maltose, pNPGlc and HML. There are several reasons for a potential inhibitory activity of APs: first, as APs are hydrolysed themselves, they act as competing substrate. Second, the cationic secondary amino group of APs may interact with carboxylate groups within the catalytic site of the enzyme, as has been shown for acarbose and other pharmaceutical glucosidase inhibitors (SIM *et al.* 2008). Finally, the

hippuryl modification of the model APs may lead to a slower release from the catalytic site due to hydrophobic interactions. This is currently under investigation with unmodified APs.

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