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Research paper

Synthesis and intestinal transport of the iron chelator maltosine in free and dipeptide form

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ABSTRACT

Maltosine, a 3-hydroxy-4-pyridinone derivative of lysine formed in the course of the advanced Maillard reaction, is an effective metal chelating agent. It therefore represents an interesting compound for the treatment of metal ion storage diseases. We synthesized 6-(3-hydroxy-4-oxo-2-methyl-4(1*H*)-pyridin-1-yl)-L-norleucine (free maltosine) and its dipeptide derivatives alanylmaltosine (Ala-Mal) and maltosinylalanine (Mal-Ala) and examined the transepithelial flux of these compounds across Caco-2 cells and their interaction with membrane transporters. Transepithelial flux of maltosine was significantly higher when added as Ala-Mal and Mal-Ala than in free form. Assays at Caco-2 cells and at HeLa cells expressing the human peptide transporter (hPEPT)1 revealed that Ala-Mal and Mal-Ala show medium to high affinity to the system. Only free but not peptide-bound maltosine inhibited the uptake of L-[^{3}H]lysine in Caco-2 and OK cells. Maltosine dipeptides were transported by hPEPT1 across cell membranes and accumulated in hPEPT1-transfected HeLa cells. In electrophysiological measurements at hPEPT1-expressing *Xenopus laevis* oocytes, Ala-Mal and Mal-Ala induced significant inward directed currents. We conclude that Ala-Mal and Mal-Ala are transported by hPEPT1 into intestinal cells and then hydrolyzed to free maltosine and alanine. The results suggest that the oral bioavailability of maltosine can be increased significantly by applying this drug candidate in peptide-bound form.

1. Introduction

Iron is an essential mineral for many metabolic functions in the organism. It is stored in ferritin and hemosiderin and circulates in plasma associated with the iron transport protein transferrin [1–3]. An abnormal accumulation of iron in the body can be caused by primary overload (hemochromatosis), by diseases such as diabetes mellitus, by alcohol abuse or by multiple blood transfusions (for example during the treatment of β-thalassemia, secondary iron overload). It is essential to eliminate excess iron from the body to prevent dysfunctions of liver, heart and endocrine glands [3,4]. Patients are usually treated with iron chelators to remove iron *via* urine and faeces. Ideally, such iron chelators are characterized by (i) efficiency, (ii) specificity, (iii) low toxicity, (iv) low molecular weight and sufficient lipophilicity, (v) slow rate of metabolism and (vi) oral bioavailability [1,5]. Desferrioxamine is used during the treatment of β-thalassemia but it is orally inactive and shows serious toxic side effects [6,7]. Therefore, a new class of orally active chelators, the hydroxypyridinones, especially the 3-hydroxy-4-pyridinones (3,4-HP), has been developed, among them deferiprone (1,2-dimethyl-3-hydroxy-4-pyridinone) [8–10]. 3,4-HP derivatives show high affinities for iron, gallium and aluminium and are resistant to enzymatic hydrolysis [7].

The 3,4-HP-derivative named maltosine (6-(3-hydroxy-4-oxo-2-methyl-4(1*H*)-pyridin-1-yl)-L-norleucine, Fig. 1) has been identified by Ledl and co-workers as a reaction product formed between the ε-amino group of lysine and carbonyl degradation products of oligosaccharides during the advanced Maillard reaction [11]. The Maillard reaction, also known as non-enzymatic glycosylation, occurs between reducing carbohydrates and lysine or arginine – as free amino acids or bound in peptides or proteins – during heating or storage of food. Some of the resulting compounds strongly bind metal ions [12–15]. Maltosine has been detected by amino acid analysis in heated milk and whey powders in concentrations of about 100 mg/kg of protein [16]. When Rehner and Walter examined the bioavailability of iron, copper and zinc in the presence of maltosine and other Maillard reaction compounds, they found that maltosine inhibited the intestinal iron uptake and increased

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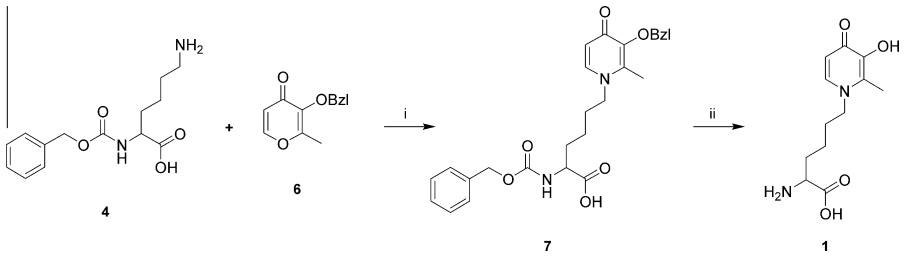

Fig. 1. Synthesis of 6-(3-hydroxy-4-oxo-2-methyl-4(1*H*)-pyridin-1-yl)-L-norleucine (maltosine) as the free amino acid. (i) Ethanol/borate buffer, pH 10.0 1/1, 80 °C, 24 h. (ii) H_2, 10% Pd/C, ethanol, rt, 18 h. **4** Z-Lys-OH, **6** 3-benzyloxy-2-methyl-4-pyrone (benzyl maltol), **7** Z-Mal(Bzl)-OH, **1** maltosine.

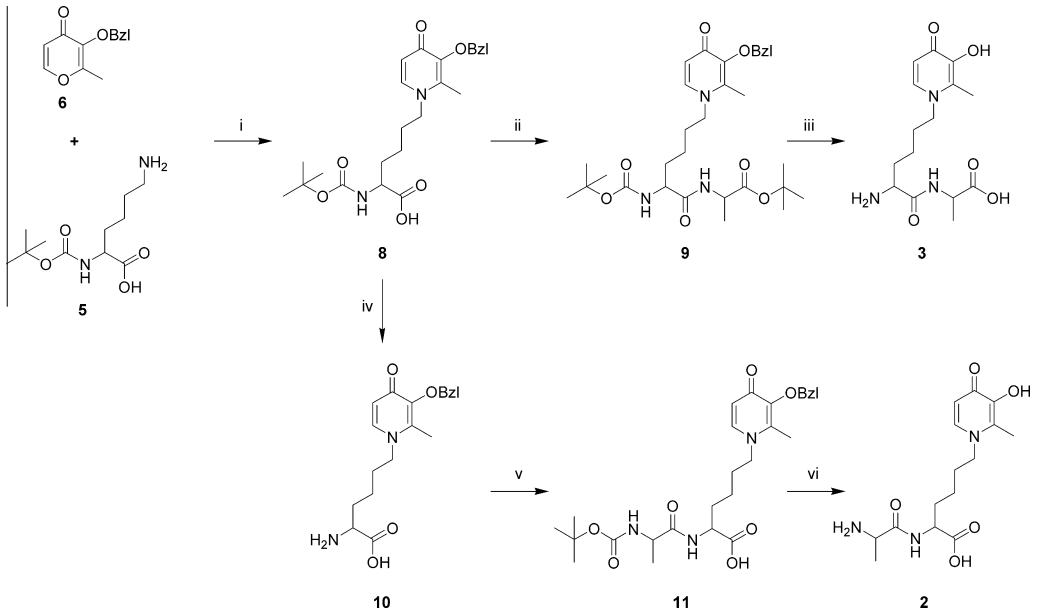

Fig. 2. Synthesis of peptide-bound 6-(3-hydroxy-4-oxo-2-methyl-4(1*H*)-pyridin-1-yl)-L-norleucine (Mal-Ala and Ala-Mal). (i) Ethanol/water 1/1, pH 13, 80 °C, 18 h. (ii) DIPEA, TSTU, DCM, rt, 30 min, then H-Ala-OBu^t, DIPEA, rt, 30 min. (iii) H_2, 10% Pd/C, methanol, rt, 18 h, then 6 N HCl/THF 1/1, rt, 60 min. (iv) 10% HOAc, 70 °C, 4 h. (v) Boc-Ala-OSu, DIPEA, DCM, rt, 18 h. (vi) H_2, 10% Pd/C, methanol, rt, 18 h, then 6 N HCl/THF 1/1, rt, 60 min. **6** 3-benzyloxy-2-methyl-4-pyrone (benzyl maltol), **5** Boc-Lys-OH, **8** Boc-Mal(Bzl)-OH, **10** H-Mal(Bzl)-OH, **9** Boc-Mal(Bzl)-Ala-OBu^t, **11** Boc-Ala-Mal(Bzl)-OH, **2** Ala-Mal, **3** Mal-Ala.

the renal iron excretion [12]. Very recent results suggest that maltosine is even more effective than the commonly used iron chelator deferiprone [unpublished data, 17].

The purpose of the present investigation was to characterize the intestinal maltosine transport and to test the hypothesis that the intestinal maltosine absorption can be increased by employing peptide-bound maltosine, e.g. alanylmaltosine (Ala-Mal) and maltosinylalanine (Mal-Ala, Fig. 2). The compounds were synthesized and characterized spectroscopically. Their total transepithelial net flux across cell monolayers was examined. In competition assays, the interaction of the compounds with the intestinal and renal lysine transporter(s) and the intestinal human peptide transporter (hPEPT)1 was studied. Experiments at hPEPT1-transfected HeLa cells and electrophysiological measurements at *Xenopus laevis* oocytes expressing hPEPT1 revealed an active, uphill and specific transport of dipeptide-bound maltosine by the intestinal peptide transporter.

2. Materials and methods

2.1. Materials

HPLC-grade acetonitrile, deuterium oxide, formic acid, unlabeled Gly-Sar, Ala-Lys and L-lysine were purchased from Sigma-Aldrich (Steinheim, Germany). *O*-(*N*-Succinimidyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TSTU) and 1-heptanesulfonic acid (sodium salt) were obtained from Molekula (Taufkirchen, Germany). Boc-Ala-OSu, 3-hydroxy-2-methyl-4-pyrone (maltol) and Z-Lys-OH **4** (Fig. 1) from Fluka (Steinheim, Germany) were used. *N*,*N*-Diisopropylethylamine (DIPEA), sodium hydroxide and trisodium citrate dihydrate were from Merck (Darmstadt, Germany). Benzyl bromide was purchased from ABCR (Karlsruhe, Germany) and H-Ala-OBu^t monohydrochloride and Lys-Ala from Bachem (Bubendorf, Switzerland). Boc-Lys-OH **5** was obtained from IRIS Biotech (Martinsried, Germany). Hydrochloric acid and palladium on activated charcoal (Pd/C, 10% w/w) were from VWR International (Darmstadt, Germany). The synthesis of 3-benzyloxy-2-methyl-4-pyrone **6** (benzyl maltol) was performed by benzylation of maltol with benzyl bromide in acetone in the presence of potassium carbonate. All other chemicals were purchased from standard suppliers and were of the highest purity available.

The cell line Caco-2 and the epithelial cervical cancer cell line HeLa were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The renal cell line OK was provided by H. Daniel (Technische Universität, Munich, Germany). Cell culture media, supplements and trypsin were purchased from Invitrogen (Karlsruhe, Germany) or PAA (Cölbe, Germany), fetal bovine serum from Biochrom (Berlin, Germany).