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Xanthophyll esters are hydrolysed in the presence of recombinant human pancreatic lipase

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Abstract

Fruit-derived bioactive xanthophyll esters have to be cleaved in the human gastrointestinal tract before absorption of free xanthophylls is possible. One candidate for ester hydrolysis is human pancreatic lipase. For estimation of their activity, an *in vitro* assay using the recombinant enzyme human pancreatic lipase (rHPL) and porcine colipase was used. Extracts of fruits were incubated with rHPL/colipase for 21 h at 37 °C. Activity of rHPL was demonstrated by an increase of free xanthophylls formed during the incubation. An extremely low activity was detected with all substrates. HPLC–(APcI)MS studies proved that lutein diesters were preferentially cleaved at the β-ionone ring. This is the first report which shows that xanthophyll esters can be cleaved in the presence of rHPL, suggesting either an unexpected secondary ester bond hydrolysis occurring within rHPL active site or, more probably, a reaction induced by other amino acids of HPL such as observed earlier with *p*-nitrophenyl acetate.

Keywords: Carotenoids; LC–MS; Recombinant human pancreatic lipase (rHPL); Xanthophyll esters

1. Introduction

Triacylglycerol digestion in humans requires pancreatic lipase (human pancreatic lipase, HPL) as a key enzyme. Since HPL is usually regarded as a 1,3-regioselective enzyme, it hydrolyzes easily primary ester groups of triacylglycerols. Secondary alcohols as 2-monoglycerides are hydrolyzed during human digestion after acyl migration of the acid from position 2 to 1 or 3 (Brockerhoff & Jensen, 1974). HPL exerts its strongest activity at the lipid–water interface of micellar or at least emulsified substrates (interfacial activation). During human digestion, generation of micelles is accomplished by various bile salts. Additional stimulation of lipolysis is caused by colipase, another enzyme synthesized by the exocrine pancreas, activating HPL in the presence of bile salts (Maylié, Charles, Gache, & Desnuelle, 1971). It is assumed that this protein allows HPL to bind to the bile salt-covered lipid–water interface. The crystal structure of the HPL–colipase complex has been published by Egloff et al. (1995).

Xanthophylls belong to the large group of secondary plant metabolites, exhibiting a wide range of biological activities. Today, it is accepted that lutein (β,ε-carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol) protect against age-related macular degeneration (AMD) and age related cataract formation (Beatty, Boulton, Henson, Koh, & Murray, 1999; Mares-Perlman, Millen, Ficek, & Hankinson, 2002), proving both xanthophylls to be ophthalmoprotective. This was confirmed by studies of Bernstein et al. (2001), in which metabolites of zeaxanthin and lutein were isolated from human retina tissue. Since the serum concentrations of lutein and zeaxanthin are influenced by the uptake (Rock et al., 2002), supply of sufficient dietary lutein and zeaxanthin is advisible. Lutein and zeaxanthin are structurally closely related. The only difference is the position of the double bond in one ionone endring: lutein exhibits one alylic double bond, whereas zeaxanthin

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features two β-ionone rings with conjugated double bonds (Fig. 1). A further xanthophyll with biological activity is β-cryptoxanthin (β,β-carotene-3-ol). During its biosynthesis in plant cells, one end ring remains unsubstituted, facilitating β-cryptoxanthin to act as vitamin-A precursor. According to Cerhan, Saag, Merlino, Mikuls, and Criswell (2003), ingestion of β-cryptoxanthin together with zinc may reduce the risk of rheumatoid arthritis. Yamaguchi (2004) proposed that β-cryptoxanthin may protect against bone diseases as osteoporosis, and Yuan, Stram, Arakawa, Lee, and Yu (2003) proposed that β-cryptoxanthin acts as a chemopreventive agent for lung cancer in humans.

Especially in tropical fruits, oranges, and red peppers, xanthophylls occur esterified with various long-chain fatty acids (Breithaupt & Bamedi, 2001; Gregory, Chen, & Philip, 1987; Mínguez-Mosquera & Hornero-Méndez, 1994). During digestion, they have to be cleaved enzymatically before absorption by entherocytes takes place. Wingerath, Stahl, and Sies (1995) investigated the carotenoid pattern in chylomicrons and serum after ingestion of tangerine juice rich in β-cryptoxanthin esters. They observed increasing amounts of free β-cryptoxanthin both in the chylomicrons as well as in the serum but no β-cryptoxanthin esters were detectable. This indicates an effective ester hydrolysis prior to incorporation into lymphatic lipoproteins. Interestingly, the enzyme responsible for hydrolysis of xanthophyll esters in the human gastrointestinal tract is actually not known (Furr & Clark, 1997). It has been assumed that HPL cleaves *inter alia* fruit-derived xanthophyll esters during digestion although these substrates exhibit esters of secondary alcohols. In former studies performed in our laboratory, no activity of HPL against xanthophyll esters was found (Breithaupt, Bamedi, & Wirt, 2002a). Lipolytic HPL activity was only detectable if retinyl palmitate was used as substrate. At that time, HPL quantities commercially available were outmost limited. The successful expression of HPL in insect cells (Thirstrup et al., 1993) and yeast (Yang & Lowe, 1998) has however allowed to obtain higher amounts of the – recombinant – human enzyme (rHPL) since a few years.

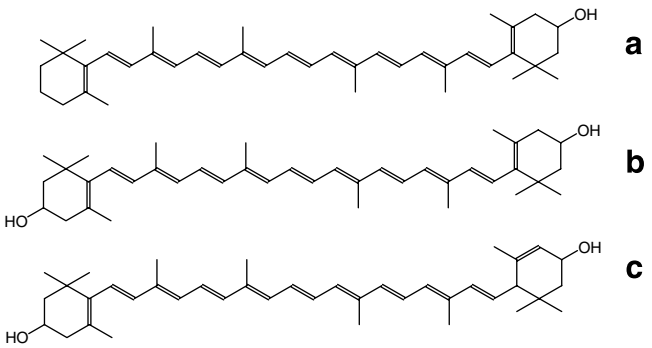

Fig. 1. Chemical structures of xanthophylls used in their acylated forms in *in vitro* rHPL-assays: (a) = β-cryptoxanthin; (b) = zeaxanthin; (c) = lutein.

For revisiting pancreatic lipase activity on xanthophyll esters, we performed an enzymatic *in vitro* assay using rHPL mixed with extracts of tangerine (*Citrus reticulata*), papaya (*Carica papaya*), wolfberries (*Lycium barbarum*) and marigold (*Tagetes erecta*) containing xanthophyll esters of β-cryptoxanthin (tangerine/papaya), zeaxanthin (wolfberries), and lutein (marigold). Since colipase was actually not available as recombinant human enzyme, porcine colipase was added to each assay.

2. Materials and methods

2.1. Chemicals and samples

Light petroleum (boiling fraction 40–60 °C), methanol, ethyl acetate and *tert*-butyl methyl ether were purchased from Merck (Darmstadt, Germany). All solvents were distilled before use. High-purity water was prepared with a Milli-Q 185 Plus water purification system (Millipore, Eschborn, Germany). Bile salts (cholic acid/deoxycholic acid sodium salt mixture 1:1) and porcine colipase (500 μg pure enzyme) were obtained from Sigma–Aldrich (Taufkirchen, Germany). Soy lecithin was a gift from Barentz GmbH, Oberhausen, Germany. β-Cryptoxanthin was generously provided by Hoffman-La-Roche (Kaiseraugst, Switzerland). Dried wolfberries (*Lycium barbarum*) were kindly provided by "Rich Nature Nutraceutical Laboraterries" WA, USA. The papaya puree (*Carica papaya*) was a gift of "Schutzgemeinschaft der Fruchtsaft-Industrie e.V. (SGF)" (Nieder-Olm, Germany) and was stored frozen at −20 °C until use. Marigold oleoresin (*Tagetes erecta*) was kindly supplied by Euram Food GmbH (Stuttgart, Germany). Direct tangerine juice (*Citrus reticulata*) was obtained from a local supermarket. Recombinant HPL (rHPL) was produced in *Pichia pastoris* using the constitutive expression system commercially available from Invitrogen GmbH (Karlsruhe, Germany) and following the experimental procedure described for the production of human pancreatic lipase-related Protein 1 (Aloulou, Grandval, De Caro, De Caro, & Carrière, 2006). The specific activity of rHPL was determined using tributyrin as substrate (pH 7.5) in presence of bile salts (0.5 mM) and porcine colipase (2:1 molar excess vs. lipase) and was found to be identical (12.500 U/mg) to that of native HPL and recombinant HPL produced in insect cells (Thirstrup et al., 1993).

2.2. Preparation of xanthophyll ester extracts

To prepare the xanthophyll ester extracts, plant samples were extracted using a ternary solvent mixture (ethyl acetate/methanol/light petroleum, 1:1:1, v/v) as follows: wolfberries (20 g) were ground using a kitchen mill. The powder was extracted using the solvent mixture until the extract was colorless. The extracts were combined (final volume: 500 mL) and the solvent evaporated in vacuum at 30 °C. To remove traces of water, ethanol (5 mL) was added