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Inhibitory effect of astaxanthin on pancreatic lipase with inhibition kinetics integrating molecular docking simulation

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ABSTRACT

Astaxanthin is a natural pigment that possesses benefit potentials to prevent obesity, however, its underlying mechanism is not clarified. In the current study, *Phaffia rhodozyma* astaxanthin was prepared and its inhibitory effect on pancreatic lipase was evaluated. The results showed that astaxanthin significantly inhibited the activity of pancreatic lipase in a dose-dependent manner within the tested concentration ranges. The kinetic analysis demonstrated that astaxanthin noncompetitively inhibited pancreatic lipase activity. In addition, astaxanthin induced secondary structure changes which resulted in decreased enzyme activities. Furthermore, the molecular docking analysis revealed that astaxanthin blocked the channel of catalytic site to delay substrate entrance or prevent product diffusion through changing the catalytic site conformation. These findings not only shed light on the underlying inhibitory mechanism of astaxanthin on pancreatic lipase, but also provide scientific evidence for extending the application of astaxanthin in functional food industries.

1. Introduction

Obesity has become a worldwide health problem due to excessive calorie intake. Obesity may induce many chronic diseases, such as cardiovascular disease, diabetes, cancer, and metabolic syndrome (Wang, Dong, Zhang, Shao, & Liu, 2014). Triglyceride is the primary energy source characterized with high calories. Synthesized and secreted by pancreas, pancreatic lipase is an important lipolytic enzyme for the triglyceride digestion. It has been reported that pancreatic lipase is capable of removing fatty acids from the α and α′ position of dietary triglycerides, yielding β-monoglycerides, long chain saturated and polyunsaturated fatty acids as lipolytic products (Mukherjee, 2003). In the small intestine, pancreatic lipase hydrolyzes triglycerides into glycerol and fatty acids, and excessive intake of which is regarded as one of the causes of obesity (Lowe, 1994). Therefore, retarding the absorption of fatty acids by inhibiting pancreatic lipase activity is considered one of the effective therapeutic approaches to prevent obesity (Ballinger & Peikin, 2002). Exploring for new natural pancreatic lipase inhibitors has gained more and more attention in the anti-obesity research fields.

Astaxanthin (3, 3′-dihydroxy-β, β′-carotene-4, 4′-dione), a water-insoluble pigment, belongs to the family of carotenoids, which is derived from both natural and synthetic sources. Since astaxanthin has two stereogenic carbon atoms at the C3 and C3′ positions, it consists of three different stereoisomers: a pair of enantiomers (3R, 3′R and 3S, 3′S) and a meso form (3R, 3′S) (Řezanka, Nedbalová, Kolouchová, & Sigler, 2013). Synthenic astaxanthin by chemical techniques (common name: chemical astaxanthin) is a stereoisomeric mixture with the ratio of (3R, 3′R)/(3R, 3′S)/(3S, 3′S) isomers in 1:2:1. However, natural astaxanthin *produced by Phaffia rhodozyma* (common name:yeast astaxanthin) is mainly 3R, 3′R isomer, and astaxanthin from *Haematococcus pluvialis* (common name:algae astaxanthin) is mainly 3S, 3′S isomer (Lim, Lee, Lee, Haam, & Kim, 2002; Sarada, Vidhyavathi, Usha, &

Abbreviations: HFFD, High-fat-high-fructose diet; *p*-NPG, 4-nitrophenyl butyrate; PNP, *p*-nitrophenol; MOPS, 3-(*N*-morpholino) propane sulphonic acid; DMSO, dimethylsulfoxide; HPLC, high performance liquid chromatography; FI, fluorescence intensity; CD spectroscopy, circular Dichroism spectroscopy; MD, molecular docking

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Ravishankar, 2006). Most commercial available astaxanthin products are derived from synthetically produced sources (Ciapara, Valenzuela, & Goycoolea, 2006). However, the stabilities and activities of synthetic astaxanthin products are lower than those of natural astaxanthins mainly extracted from *P. rhodozyma* and *H. pluvialis* (Dong, Zhao, Ma, Xing, & Sun, 2006). Furthermore, compared to *H. pluvialis* originated astaxanthin, astaxanthin obtained from *P. rhodozyma* is more common without heavy metal contaminates. Particularly, *P. rhodozyma* has higher biomass production with rapid self-propagation and can produce 3R, 3′R configuration astaxanthin, which represents more than 80% of the total carotenoids produced (Bhatt, Ahmad, & Panda, 2013). It has been reported that astaxanthin possesses numerous biological activities, such as antioxidant (Ambati, Moi, Ravi, & Aswathanarayana, 2014), anti-inflammatory (Bhuvaneswari, Yogalakshmi, Sreeja, & Anuradha, 2014), anti-cancer (Rao et al., 2013), anti-diabetic (Sila et al., 2015), as well as cardiovascular disease preventing activities (Augusti et al., 2012), Among these activities, the potential antioxidant activity of astaxanthin is most attractive due to that antioxidant activity has a significant role in the protection against oxidation-damages, inflammation, cancer, aging and age-related diseases, as well as the promotion of the immune response, and the promotion and maintenance of the function of important organs including heart, liver, eye, arthrosis and prostate (Guerin, Huntley, & Olaizola, 2003). Moreover, the antioxidant activity of astaxanthin is approximately 10 times more effective than those generated by other carotenoids including zeaxanthin, lutein, canthaxanthin, and β-carotene, and approximately 100 times higher than that induced from α-tocopherol (Miki, 1991). Recently, Liu et al. reported that the three astaxanthin stereoisomers showed significantly different activities. 3S, 3′S astaxanthin isomer (from *H. pluvialis*) exhibited the strongest antioxidant and anti-aging activities both *in vitro* and *in vivo*, followed by 3R, 3′R astaxanthin (from *P. rhodozyma*) and synthenic astaxanthin (Liu et al., 2016). Therefore, astaxanthin has been considered to be a promising commercial antioxidative compound and widely applied in food, aquaculture and pharmaceutical industries (Tominaga, Hongo, Karato, & Yamashita, 2012).

Natural astaxanthin was capable of inhibiting the increases of body weights and adipose tissue weights in mice fed a high-fat diet by stomach intubation without undesirable side effects and toxicity. Meanwhile, the mouse liver weight, liver triglyceride, plasma triglyceride, and total cholesterol were reduced (Ikeuchi, Koyama, Takahashi, & Yazawa, 2007). Bhuvaneswari et al. also found natural astaxanthin could improve liver function and limit lipid accumulation in liver of the high-fat-high-fructose diet (HFFD) -fed mice without any toxic effects (Bhuvaneswari, Arunkumar, Viswanathan, & Anuradha, 2010). According to these findings, astaxanthin is considered to be the potential safe and effective natural therapeutic compound to prevent obesity. Although piling evidence has indicated the beneficial effects of astaxanthin in obesity control, the underlying mechanism of the anti-obesity potential of astaxanthin remain to be elucidated. Limited information is known about the inhibitory effects of astaxanthin against pancreatic lipase. Moreover, neither is the anti-obesity potential of astaxanthin derived from *P. rhodozyma*. Therefore, in this study, we prepared astaxanthin from *P. rhodozyma* and evaluated the inhibitory effects of *P. rhodozyma* astaxanthin, chemosynthetic astaxanthin, and *H. pluvialis* astaxanthin on pancreatic lipase, and attempted to explain the possible anti-obesity mechanism of astaxanthin as enzyme inhibitors. We found that among these astaxanthins, *P. rhodozyma* astaxanthin demonstrated the strongest inhibitory effect on pancreatic lipase. The inhibition kinetics of *P. rhodozyma* astaxanthin on pancreatic lipase was further investigated and integrated with molecular docking simulations to analyze the binding behaviors of astaxanthin to pancreatic lipase. The information obtained in present study may shed light on extending the application of *P. rhodozyma* astaxanthin in functional food industries.

2. Materials and methods

2.1. Materials

The thalli of *P. rhodozyma* were provided by Xiamen Huisheng Biology Co., Ltd (Xiamen, China). Pancreatic lipase (from porcine pancreas, 100–400 units/mg), 4-nitrophenyl butyrate (*p*-NPB), *p*-nitrophenol (PNP), 3-(*N*-morpholino) propanesulphonic acid (MOPS), orlistat, synthenic astaxanthin (100%, United States Pharmacopeia Reference Standard (USP)), and *H. pluvialis* originated astaxanthin (≥97%, HPLC), methanol (for HPLC, ≥99.9%) were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Acetone, petroleumether, ethylacetate, methanol, dichloromethane, dimethylsulfoxide (DMSO), tris-base, and Ethylene Diamine Tetraacetic Acid (EDTA) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). For convenience, *P. rhodozyma* originated astaxanthin, synthetic astaxanthin, and *H. pluvialis* originated astaxanthin were named yeast astaxanthin, chemical astaxanthin, and algae astaxanthin, respectively.

2.2. Preparation of P. rhodozyma astaxanthin

The thalli of *P. rhodozyma* (100 g) were added to 200 mL of DMSO (used as the disruption reagent for *P. rhodozyma* cell wall) at 52 °C and agitated vigorously for 10 min to release the astaxanthin (Du et al., 2016). Subsequently, 1500 mL of acetone was added and stirred for 5 min to extract the astaxanthin. After centrifugation at 5000*g* for 10 min, the supernatant was separated and concentrated under a vacuum condition at 32 °C until acetone was evaporated thoroughly to afford DMSO fraction. Then petroleum ether was further applied to remove the lipids and low polar carotenoids contained in DMSO fraction, and the bottom phase was further extracted with saturated sodium chloride solution and ethyl acetate. Upon agitating and layering, the ethyl acetate phase was collected and concentrated by evaporating at 32 °C to yield a dark red residue, followed by re-crystallization in dichloromethane/methanol (1/20, v/v) at least twice to obtain astaxanthin. The purity of isolated astaxanthin was analyzed by the high performance liquid chromatography (HPLC) according to the method of Du et al. (2016). And HPLC analysis revealed the separated astaxanthin with purity 94.54%.

2.3. Lipase activity assay

The inhibitory effects of astaxanthins (yeast astaxanthin, chemical astaxanthin, algae astaxanthin) on pancreatic lipase were evaluated according to the method described by Kim et al. (2007) with some modifications. Briefly, the enzyme-buffer solutions were prepared by adding 60 μL pancreatic lipase solutions (10 units in 10 mM MOPS containing 1 mM EDTA, pH 6.8) to 1.7 mL Tris-buffer (100 mM Tris-HCl containing 5 mM $CaCl_2$, pH 7.0). Then, 200 μL astaxanthins (dissolved in DMSO) were mixed with the enzyme-buffer solutions to final concentration of 0, 5, 10, 30, 40, 50 or 70 μg/mL, respectively. After pre-incubation at 37 °C for 15 min, 40 μL *p*-NPB (6 mM in DMSO) used as the substrate solution was added to each astaxanthin-lipase reaction mixture and further incubated at 37 °C for 15 min. Finally, the reaction was terminated by heating at 100 °C for 5 min and then cooled down to room temperature. The absorbance of each reaction mixture was measured at 405 nm using a Unico®7200 spectrophotometer (Unico Instrument Corporation, Dayton, NJ, USA). The reaction mixture without lipase was used as a negative control. The IC_{50} value was defined as the concentrations of astaxanthins required to reach 50% inhibition of lipase activity and estimated by linear fitting. A semi synthetic pharmacological inhibitor of lipase, orlistat, served as a positive control at 0.05 μg/mL and its inhibitory effect on pancreatic lipase was tested in the preliminary experiment under the same testing conditions. The inhibitory rates of astaxanthins on pancreatic lipase were calculated according to the following equation: