Food Chemistry 149 (2014) 99–106

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Lignin binding to pancreatic lipase and its influence on enzymatic activity

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ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form 5 September 2013
Accepted 15 October 2013
Available online 28 October 2013

Keywords:
Pancreatic lipase
Lignin
Lignin–pancreatic lipase complex

ABSTRACT

In this paper, we find that the effect of lignin on pancreatic lipase (PL) is dependent on reaction medium and substrate used. Experimental results reveal that lignin can gradually bind to PL to form a PL–lignin complex, resulting in an increased activity of the enzyme. The binding process is spontaneous and the PL–lignin complex formation is an endothermic reaction induced by hydrophobic and electrostatic interaction. There is a non-radiation energy transfer from PL to lignin during the binding process, and the binding of lignin to PL conforms to a secondary exponential decay function. Moreover, the α-helix content of the enzyme will be changed and the rigidity of its side chain will be enhanced due to the formation of lignin–PL complex. This study has not only provided the activation effect of lignin on PL, but also given an insight into the interaction between lignin and the enzyme, which would benefit the application of lignin in the pharmacy and food industry, as well as other fields.

1. Introduction

A major enzyme found in humans and other mammals, lipase, digests fats, including triacylglycerol and phospholipids. Pancreatic lipase (PL), the principal lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient digestion of triglycerides. It removes fatty acids from the α and α′ position of dietary triglycerides, yielding β-monoglycerides and long chain saturated and polyunsaturated fatty acids as the lipolytic products (Mukherjee, 2003; Shi & Burn, 2004; Thomson, DePover, Keelan, JarockaCyrta, & Clandinin, 1997). PL is responsible for the hydrolysis of 50–70% total dietary fats (Birari & Bhutani, 2007). The three-dimensional structure of PL has been studied by X-ray crystallography. It is a single-chain glycoprotein of 449 amino acids and is divided into two folding units: a larger amino-terminal domain containing 1–336 residues and a smaller carboxy-terminal domain at residues 337–449. The active site is a triad comprising His-263, Asp-176 and Ser-152 in the larger amino-terminal domain (van Tilbeurgh, Sarda, Verger, & Cambillau, 1992; Winkler, D'Arcy & Hunziker, 1990). Serum PL is associated with many kinds of diseases. For example, PL is secreted into duodenum, and normally its concentration in serum is very low. When the pancreas suffers pancreatitis or pancreatic adenocarcinoma, the pancreas may begin autolysis, which leads to the elevation of serum PL (Koop, 1984). Moreover, the elevation of PL can also be seen in peptic ulcer perforation, intestinal obstruction and acute cholecystitis. On the other hand, in the later stage of chronic pancreatitis, the acini organisation is seriously destroyed, and the enzyme secreted into the cycle is decreased, thus serum PL is decreased. In addition, pancreatic fibrocystic disease may lead to the decrease of PL in duodenum.

Dietary fibre is a part of plant material in the diet, which is resistant to enzymatic digestion. It includes cellulose, noncellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilages and a non-carbohydrate component lignin (Dhingra, Michael, Rajput, & Patil, 2012). As a kind of dietary fibre, lignin is a random copolymer consisting of phenylpropane units with characteristic side chains. It is one of the most important bio-resources distributed in plant organs. It is formed by the dehydrogenative polymerisation of three hydroxycinnamyl alcohols: p-coumaryl, coniferyl, and sinapyl alcohols, giving rise to different types of lignin units called p-hydroxyphenyl, guaiacyl, and syringyl. As an important part of healthy diet, lignin is widely spread in fruits, vegetables and grains. It can regulate food intake and digestion, improve bowel function and alleviate constipation in patients (Takahashi et al., 1994). It also has diverse pharmacological activities such as antitumor, anti-microbial, anti-HIV and antioxidant activities (Sakagami, Kushida, Oizumi, Nakashima, & Makino, 2010). Moreover, it has various therapeutic functions, for instance, reducing risk of heart disease, lowering variance in blood sugar levels, etc. (Dhingra et al., 2012).

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http://dx.doi.org/10.1016/j.foodchem.2013.10.067

Our recent studies have revealed that lignin can be a new kind of activator to greatly increase α-amylase activity. This finding has not only offered new insights for the exploration of an activator of α-amylase, but also contributed to the application of lignin in the food industry (Zhang, Cui, Yin, Sun, & Li, 2013). The aim of this work is to assess the effect of lignin on PL activity. Firstly, the effect of lignin on the enzymatic activity of PL has been studied. Secondly, the mechanism of interaction between PL and lignin is figured out by fluorescence spectroscopy, UV–visible spectroscopy, circular dichroism and atomic force microscopy (AFM). Finally, the biological significance of the effect of lignin on PL has been discussed.

2. Materials and methods

2.1. Materials and reagents

Pancreatic lipase (PL) (L3126, type II from porcine pancreas), casein, trizma-base, sodium acetate and lignins with different molecular weight (8000, 10,000, 12,000, 18,000, 52,000, 54,000 Da) were purchased from Sigma (USA). Triton X-100 and triolein were purchased from Sangon Biotech. (Shanghai). Isooctane, *p*-nitrophenyl laurate (pNP laurate) and cupric acetate were purchased from Aladdin Reagent (Shanghai). HCl, pyridine and phosphatidylcholine were purchased from Sinopharm Chemical Reagent (Shanghai). All the reagents were analytical or of chromatographic purity. Water was doubly distiled and deionised.

2.2. Enzymatic activity assay

Method 1: The lipase activity was tested by measuring the fatty acids released from emulsified triolein (Kwon & Rhee, 1986). The emulsions were prepared by dissolving 2.4 g casein and 200 mg phosphatidylcholine in a beaker with 100 ml of 100 mM Tris–HCl (pH 8.2). The mixture was diluted to 200 ml with Tris–HCl. The substrate emulsion was prepared by mechanical stirring of 200 μl triolein and 10 ml emulsion. Lipase was dissolved in Tris–HCl buffer at 1 mg/ml and the supernatant was used after centrifugation at 6000 rpm (3500*g*) for 10 min. The assay was carried out in a total volume of 700 μl containing 100 μl of Tris–HCl buffer and lignin with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0 mg/ml), 400 μl of substrate emulsion, and 200 μl of lipase solution (90 units/ml). After incubation for 15 min at 37 °C, the reaction was stopped by the addition of 200 μl of HCl. Then, 300 μl of isooctane was added. The upper layer was collected, followed by the addition of 400 μl copper reagent (5% copper acetate, pH 6.1 regulated by pyridine). After vigorously stirring for 10 min, the tube was centrifuged and the upper aqueous phase was collected by suction and then measured at 720 nm against a reagent blank.

Method 2: Lipase activity was evaluated by the hydrolysis of *p*-nitrophenyl laurate (pNP laurate) spectrophotometrically recorded at 405 nm (McDougall, Kulkarni, & Stewart, 2009). The substrate solution was prepared through dissolving pNP laurate (0.08% w/v) in 5 mM sodium acetate buffer (pH 5.0) containing 1% Triton X-100. After heating in a boiling water bath for 1 min, the solution was cooled to room temperature. Lipase was dissolved in Tris–HCl buffer at 1 mg/ml and the supernatant was used after centrifugation at 6000 rpm (3500*g*) for 10 min. The assay was carried out in a total volume of 1000 μl containing 300 μl of Tris–HCl buffer and 100 μl of lignin with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0 mg/ml), 400 μl of substrate emulsion, and 200 μl of lipase solution (90 units/ml). The blank solution contained 400 μl buffer, 400 μl substrate solution and 200 μl lipase. After mixing with enzyme, lignin and substrate solution, the mixture was incubated at 37 °C for 20 min and heated in a boiling water bath for 10 min to stop the reaction. Then samples were centrifuged at 6000 rpm (3500*g*) for 10 min and determined at 400 nm against the blank in a UV–visible spectrophotometer (Shimadzu Co., Kyoto, Japan).

2.3. Fluorescence quenching

Fluorescence measurements were performed on a spectrofluorimeter Model LS-55 (PerkinElmer, USA) spectrometer, equipped with a thermostated sample compartment, in conjunction with a circulating bath (Lauda, K-2R, Brinkmann Instruments, Westbury, NY, USA). Lignin with molecular weight of 8000 Da was selected for fluorescence analysis and the other works. 2.0 ml of 5 μM lipase solution in 1.0 cm quartz cells was titrated by successive additions of 0.05 mM lignin solution at 0, 10, 10, 20, 20, 40, 40 and 60 μl. Fluorescence emission spectra of lipase were recorded over a wavelength range of 300–500 nm with excitation wavelength at 280 and 295 nm, at varying temperature (297, 307 and 317 K). Both excitation and emission bandwidths were set at 10 nm. The synchronous fluorescence spectra were recorded in the range of 200–600 nm with $\Delta\lambda$ at 30 and 60 nm. Moreover, 1 ml of 2 μM PL solution was separately mixed with 1 ml of 5 and 0.5 μM lignin, and kinetic fluorescence spectra of the mixture were continuously tested for 1 h with the 1 min interval when the excitation wavelength was set at 295 nm.

The intrinsic fluorescence has been analysed according to the Stern–Volmer equation (Kandagal et al., 2006):

$$F_0/F = 1 + k_q \times \tau_0 \times [\text{lignin}] = 1 + K \times [\text{lignin}] \quad (1)$$

where F_0 and F are the fluorescence emission intensities without and with lignin; [lignin] is lignin concentration; k_q is the fluorescence quenching rate constant; τ_0 is the fluorophore fluorescence lifetime without quencher; and K is a constant, equal to the reciprocal of the quencher concentration when the fluorescence intensity decreases by half.

For static quenching, the binding constant (Puida, Ivanauskas, Ignatjev, Valincius, & Razumas, 2008) and the number of binding sites (n) of static quenching can be calculated using the following equation:

$$\lg[(F_0 - F)/F] = \lg K_s + n \ \lg [\text{lignin}] \quad (2)$$

The thermodynamic parameters enthalpy (ΔH), entropy change (ΔS) and free energy (ΔG) are calculated by the Van't Hoff equation:

$$\ln K_s = \Delta H/RT + \Delta S/R \quad (3)$$

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

where K_s is the binding constant at the corresponding temperature and R is the gas constant.

The energy transfer efficiency E, the distance r and the critical energy transfer distance R_0 are calculated by the following equations (Sklar, Hudson, & Simoni, 1977):

$$E = 1 - F/F_0 = R_0^6/(R_0^6 + r^6) \quad (5)$$

$$J = \int F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda \quad (6)$$

$$R_0^6 = 8.8 \times 10^{-25} K^2 n^{-4} \Phi_D J \quad (7)$$

where r represents the distance between a donor and an acceptor, R_0 is the critical distance at which transfer efficiency equals to 50%, K^2 is the spatial orientation factor of the dipole, n is the refrac-