Carbohydrate Polymers 201 (2018) 65–74

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

A pectin from fruits of *Lycium barbarum* L. decreases β-amyloid peptide production through modulating APP processing

Lishuang Zhou[a,b,c,1], Wenfeng Liao[a,b,1], Hui Zeng[a,b], Yanli Yao[a,b], Xia Chen[a,b], Kan Ding[a,b,c,*]

[a] Glycochemistry and Glycobiology Lab, Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, PR China
[b] University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, PR China
[c] School of Pharmacy, Zunyi Medical University, 201 Dalian Road, Zunyi 563000, PR China

ARTICLE INFO

Keywords:
Lycium barbarum L.
Pectin
$A\beta_{42}$
Alzheimer's disease
Amyloid precursor protein processing

ABSTRACT

Here, a pectin LBP1C-2 with the molecular weight of 99.8 kDa was purified from fruits of *Lycium barbarum* L. Its structure was elucidated as a backbone of alternate 1, 2-linked α-Rha*p* and 1, 4-linked α-Gal*p*A, with branches of terminal (T) -, 1, 3-, 1, 6- and 1, 3, 6-linked β-Gal*p*, T-, 1, 5- and 1, 3, 5-linked α-Ara*f* and T-linked β-Rha*p* substituted at C-4 of 1, 2, 4-linked α-Rha*p*. The cell-based experiments indicated that LBP1C-2 suppressed $A\beta_{42}$ production in a dose-dependent manner with no cytotoxicity. Further study showed that expression of β-site APP cleaving enzyme 1 (BACE1) was attenuated by LBP1C-2, while expression of ADAM10 was up-regulated by LBP1C-2. Moreover, LBP1C-2 promoted the expression of insulin-degradation enzyme (IDE). These results suggested that LBP1C-2 might be a leading compound for anti-Alzheimer's disease therapy by decreasing $A\beta_{42}$ production through mediating BACE1 and ADAM10 as well as IDE expression.

1. Introduction

Alzheimer's disease (AD), which is the most commonform of dementia, is a chronic neurodegenerative brain disorder characterized by progressive cognitive impairment and memory deterioration (Scheltens et al., 2016). Senile plaques and intracellular neurofibrillary tangles are two dominant hallmarks of AD pathological features (Zhu et al., 2013). Genetic and biochemical evidences demonstrate that physiologic generation of the neurotoxic β-amyloid peptide (Aβ) from sequential proteolysis of amyloid precursor protein (APP) is the vital process in the development of AD (O'Brien & Wong, 2011). Full-length APP is a single-pass transmembrane protein, which is synthesized in the endoplasmic reticulum (ER) and then transported through the Golgi apparatus to the trans-Golgi-network (TGN) (Hartmann et al., 1997; Xu et al., 1997). Generally, two major pathways are involved in APP processing: the initial cleavage of β-secretase and intramembrane cleavage of γ-secretase mediated amyloidogenic pathway which produces Aβ peptide, the α- and γ-secretase mediated non-amyloidogenic pathway, which cleaves APP within the Aβ sequence and releases a large soluble ectodomain of APP named sAPPα (Haass & Selkoe, 2007; Thinakaran & Koo, 2008). Thus, targeting modulating the APP processing has been considered as one of the prospective strategies in anti-AD research.

Total Aβ level is not only dependent on the proteolytic activities of the APP cleaving secretases, but also on Aβ degradation. There are three major pathways by which Aβ is cleared from the brain: through proteolytic degradation by a mass of proteases including neprilysin (NEP) and insulin-degrading enzyme (IDE), through lysosomal degradation in brain parenchyma cells such as astrocytes, neurons and microglia and the cerebrovascular system-mediated clearance pathway by the interstitial fluid (Saido & Leissring, 2012).

Lycium barbarum is a multi-branched defoliated shrubbery belonging to the solanaceae family and widely distributed in arid and semi-arid regions of Northwestern China (Hu, Qu, Chu, Li, & He, 2017). As a well-known traditional Chinese herbal medicine, L. *barbarum* has been used historically to improve vision, moisten lung and nourish the liver and kidney through balancing "ying" and "yang" in the body for thousands of years (Chiu et al., 2010). L. *barbarum* constitutes with multitudinous components, such as polysaccharides, betaine, taurine, cerebroside, vitamin, flavonoids, fatty acids and so on (Lam, Tipoe, So, & Fung, 2015). As the major active ingredient of L. *barbarum* fruit, polysaccharide has been reported to possess a wide range of biological activities including antivirus, antioxidant, reducing blood glucose and

* Corresponding author at: Glycochemistry and Glycobiology Lab, Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, PR China.
E-mail address: dingkan@simm.ac.cn (K. Ding).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.carbpol.2018.08.050
Received 4 July 2018; Received in revised form 10 August 2018; Accepted 10 August 2018
Available online 15 August 2018
0144-8617/

neuroprotective properties (Cheng et al., 2015). Recent studies have concerned the neuroprotective effect of L. *barbarum* polysaccharides (LBPs) in some neuronal diseases such as retinal disorders, stroke, spinal cord and ischemic injury and Alzheimer's disease (Shi et al., 2017). So far, LBPs have been proved to alleviate or delay several key processes during AD progression by ameliorating the Aβ-induced neurotoxicity, playing beneficial roles in aging-induced AD symptoms and preserving memory functions in animal models (Xing, Liu, Xiao, & So, 2016). However, in most research, the detailed structure features of LBPs and their biological activities as well as its underling mechanism of active substance were not fully expounded.

In this study, we firstly reported the structure of the pectin named LBP1C-2 from fruits of L. *barbarum*. Then, we investigated the effect of LBP1C-2 on $A\beta_{42}$ production *in vitro* and the possible underlying molecular mechanism of this action. To the best of our knowledge, this was the first report about the homogeneous polysaccharide isolated from L. *barbarum* which might block $A\beta_{42}$ secretion directly in Alzheimer's disease.

2. Materials and methods

2.1. Materials

The fruits of L. *barbarum* (5.0 kg) were purchased from shanghaikangqiao Co., Ltd., (Shanghai, China). The plant was cultivated in Ningxia Huizu Autonomous Region, P. R. China. Food-grade enzymes (cellulase (U = 2000), papain (U = 400,000) and amylase (U = 1,000,000)) were purchased from Shandong Lonct Enzymes Co., Ltd, (Linfen, Shandong, China) and Nanning Pangbo Biological Engineering Co., Ltd., (Nanning, Guangxi, China), respectively. DEAE Sepharose™ Fast Flow and Sephacryl S-300 HR columns were purchased from GE Healthcare. 1-phenyl-3-methyl-5-pyrazolone (PMP) and trifluoroacetic acid (TFA), Geneticin (G418), MTT, cocktail and poly-L-lysine were from Sigma-Aldrich (Saint Louis, USA). 1-Cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate (CMC) was purchased from Tokyo Chemical Industry Co., Ltd, (Tokyo, Japan).

2.2. Extraction, isolation and purification of LBP1C-2

Crude polysaccharides LBP1 were extracted and isolated from fruit of L. *barbarum* as the previous report (Zhou, Huang, Yue, & Ding, 2018; Zhou et al., 2018). In briefly, the fraction of LBP1C was pooled from the 0.2 M aqueous NaCl by DEAE Sepharose™ Fast Flow column and further purified by Sephacryl S-300 HR column to give one target polysaccharide LBP1C-2.

2.3. Homogeneity and molecular weight

Homogeneity of polysaccharide LBP1C-2 was examined by high-performance gel permeation chromatography (HPGPC) using the Agilent 1260 HPLC system fitted with the GPC software, equipped with tandem Waters UItrahydrogel ™ 2000 and UItrahydrogel ™ 500 columns described in a previous report (Yue, Liu, Qu, & Ding, 2017).

2.4. Monosaccharide composition analysis

The glycosyl composition was analyzed using HPLC method for PMP pre-column derivation method as precious reported (Cong, Xiao, Liao, Dong, & Ding, 2014). In briefly, LBP1C-2 (2 mg) was hydrolyzed with 4 mL 2 M trifluoroacetic acid (TFA), followed by PMP derivation. 10 μL of the derivative solution was injected into HPLC system to be analyzed.

2.5. Linkage patterns analysis

The polysaccharide (LBP1C-2) was methylated according to the literature with slight modification (Ciucanu & Kerek, 1984; Nie et al., 2011). Briefly, the sample LBP1C-2 (10 mg) was dissolved in 2 mL dimethyl sulfoxide (DMSO) at room temperature, and stirred vigorously with 30 mg anhydrous NaOH powder for 1.5 h under nitrogen. Iodomethane (0.5 mL) was added dropwise under ice bath and continually stirred for 1.5 h at room temperature in dark place. Then, 1 mL deionized water was added to quench the reaction. Finally, the mixture was extracted with $CHCl_3$ and water (1:1, v/v) for three times, and the organic phase was dried by $MgSO_4$ to obtain the methylated polysaccharide. The above experiment was repeated for once. The resulting product was transformed into partially methylated alditol acetate for GC–MS analysis.

2.6. Partial acid hydrolysis

LBP1C-2 (200 mg) was incubated in 0.2 M TFA (20 mL) at 100 ℃. The hydrolysate solution was dialyzed against distilled water (MWCO 3500 Da) for 24 h. The retentate LBP1C-202I was collected and lyophilized.

2.7. NMR analysis

The deuterium-exchanged polysaccharides were prepared for NMR analysis. ^1H NMR, ^{13}C NMR were measured using Bruker AVANCE III NMR spectrometer, which was operating at 500 MHz at 25 ℃ with a cryogenic NMR probe. Acetone was employed as an internal standard (2.29 for hydrogen, 31.5 for carbon) for the chemical shifts reference in ^1H and ^{13}C NMR spectra.

2.8. Uronic acid reduction

40 mg polysaccharide (LBP1C-2) was dissolved in 40 mL distilled water and then 600 mg CMC was added. The pH was kept at 4.75 with 0.01 M HCl for 2 h. Then 16 mL of 2 M fresh sodium borohydride ($NaBH_4$) solution was added slowly and the pH of mixture was controlled at 7 with 4 M HCl for 2 h. Finally, the reaction mixture was dialyzed against deionized water (MWCO 3500 Da) for 24 h, the retentate was freeze-dried. The aforementioned process was repeated twice.

2.9. Cell lines and culture conditions

Chinese hamster ovary (CHO) cells stable-transfected amyloid precursor protein (APP) and β-site APP cleaving enzyme 1 (BACE1) (CHO/APPBACE1) were maintained in the lab and grown in Ham's F12 medium (Hyclone, USA) supplementing 10% (v/v) fetal bovine serum (FBS) (Gibco, USA) and antibiotics (100 μg/mL streptomycin and 100 U/mL penicillin, Gibco, USA). Human embryonic kidney (HEK293) cells transfecting with APP Swedish mutant$^{K595N/M596L}$ (HEK293-APPsw) (kindly provided by Prof. Haiyan Zhang, Shanghai Institute of Material Medica, China) were cultured in Dulbecco's modified Eagle's medium (DMEM medium) containing 10% FBS, 200 μg/mL G418 (Geneticin) and antibiotics. SHSY5Y cells were purchased from the Cell Bank in the Type Culture Collection Center in Chinese Academy of Sciences (Shanghai, China), and grown in a mixture of MEM and F12 medium (v/v = 1:1) supplementing with 10% FBS and antibiotics. All the cells were maintained at 37 ℃ in a humidified incubator with 5% CO_2.

2.10. ELISA assay

HEK293-APPsw and CHO/APPBACE1 cells were incubated with LBP1C-2 or without (Control group) for 24 h. Subsequently, the cell culture media was collected and the protease inhibitor cocktail was added, the supernatant of the medium was collected after the centrifugation by 20,000*g* for 10 min at 4 ℃. ELISA kit of $A\beta_{42}$ (Invitrogen, USA) was employed to evaluate the contents of $A\beta_{42}$ in each group.