Biochimica et Biophysica Acta 1831 (2013) 698–708

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Cyanidin is an agonistic ligand for peroxisome proliferator-activated receptor-alpha reducing hepatic lipid

Yaoyao Jia [a,b], Jin-Young Kim [a,b], Hee-jin Jun [a,b], Sun-Joong Kim [a], Ji-Hae Lee [a,b], Minh Hien Hoang [a,b], Hyun Sook Kim [a], Hyo Ihl Chang [a], Kwang-Yeon Hwang [a], Soo-Jong Um [c], Sung-Joon Lee [a,b,*]

[a] Department of Biotechnology, the Graduate School of Biotechnology, Korea University, Seoul 136-713, Republic of Korea
[b] Division of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea
[c] Department of Bioscience and Biotechnology, Sejong University, Seoul 143-747, Republic of Korea

ARTICLE INFO

Article history:
Received 17 August 2012
Received in revised form 15 November 2012
Accepted 29 November 2012
Available online 8 December 2012

Keywords:
Cyanidin
Hepatocyte
Lipid metabolism
PPAR

ABSTRACT

To investigate the underlying mechanism of targets of cyanidin, a flavonoid, which exhibits potent anti-atherogenic activities *in vitro* and *in vivo*, a natural chemical library that identified potent agonistic activity between cyanidin and peroxisome proliferator-activated receptors (PPAR) was performed. Cyanidin induced transactivation activity in all three PPAR subtypes in a reporter gene assay and time-resolved fluorescence energy transfer analyses. Cyanidin also bound directly to all three subtypes, as assessed by surface plasmon resonance experiments, and showed the greatest affinity to PPARα. These effects were confirmed by measuring the expression of unique genes of each PPAR subtype. Cyanidin significantly reduced cellular lipid concentrations in lipid-loaded steatotic hepatocytes. In addition, transcriptome profiling in lipid-loaded primary hepatocytes revealed that the net effects of stimulation with cyanidin on lipid metabolic pathways were similar to those elicited by hypolipidemic drugs. Cyanidin likely acts as a physiological PPARα agonist and potentially for PPARβ/δ and γ, and reduces hepatic lipid concentrations by rewiring the expression of genes involved in lipid metabolic pathways.

1. Introduction

The peroxisome proliferator-activated receptor (PPAR) is a nuclear hormone receptor, which is made up of transcriptional factors activated by the binding of ligands to their ligand-binding domains (LBDs) [1]. Although the three PPAR subtypes, alpha (α), delta/beta (δ/β), and gamma (γ), display different cellular distributions and distinct pharmacological profiles, they are all essentially related to lipid and glucose metabolism [2]. Because PPARs share similar structures within the LBDs, synthetic ligands that simultaneously activate at least two of the PPAR subtypes represent potent candidate drugs for the treatment of abnormal metabolic homeostasis by improving clinical symptoms of cardiovascular disease, type 2 diabetes mellitus (T2DM), and obesity [3,4].

PPAR activation provides a number of metabolic benefits. However, potent full agonists can induce significant side effects. For example, rosiglitazone, induces carcinogenesis, edema, and cardiovascular complications, and thus its use in newly diagnosed T2DM has been suspended since 2010 [5]. In addition, the recent focus in this area has centered on the concept of selective PPAR modulators, particularly for PPARγ, following the observation that the full agonists such as rosiglitazone and pioglitazone are counterbalanced by receptor-mediated side effects [6]. A partial agonist could retain efficacy but reduce the transcriptional regulation thought to be responsible for the attendant side effects.

Considering the beneficial pharmacological effects of the PPAR subtypes, the concept of simultaneously but moderately activating all PPAR subtypes with a single compound, for example, pan- or dual-agonists, could be advantageous for the prevention and treatment of the clinical symptoms of ectopic fat accumulation, including liver steatosis, diabetes mellitus, and metabolic syndrome. Simultaneous activation of all PPAR subtypes may also reduce the occurrence of adverse side effects, which are often associated with full PPAR agonists [7].

Initially, we screened approximately 900 Korean natural extracts and compounds from plant and marine organisms for PPAR agonist or antagonist activity using a reporter gene assay, and found that cyanidin exhibited potent PPAR activity. Cyanidin is a well-defined anthocyanidin abundant in fruits, and vegetables [8] with various biological activities, including potent antioxidant, radical-scavenging, hypolipidemic, and anti-inflammatory activities. Therefore, cyanidin protects against cellular oxidative damage and reduces plasma and

Abbreviations: PPAR, peroxisome proliferated-activated receptor; LBD, ligand-binding domain; RXR, retinoid X receptor; Apo, apolipoprotein; K_D, equilibrium dissociation constant; EC_{50}, the half maximal effective concentration; SPR, surface plasmon resonance; TR-FRET, time-resolved fluorescent resonance energy transfer; PCA, procatechic acid; PGA, phloroglucinaldehyde

* Corresponding author at: Department of Biotechnology, the Graduate School of Biotechnology, Korea University, Seoul, 5-ga, Anam, Seongbuk, Seoul, Republic of Korea. Tel.: +82 2 3290 3029; fax: +82 2 3290 3653.
E-mail address: junelee@korea.ac.kr (S.-J. Lee).

1388-1981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbalip.2012.11.012

cellular accumulation of undesirable lipids, collectively reducing the risk of age-related metabolic diseases, such as cardiovascular diseases, diabetes mellitus, and obesity [9].

Some evidence also suggests that foods with cyanidin and its glycosides inhibit the formation of atherogenic lipoprotein and oxidized low-density lipoprotein (LDL) particles *in vitro* [10,11] and *in vivo* [12–15]. Several studies have shown that cyanidin-rich diet results in significantly reduced cholesterol, triglyceride (TG), and ApoB plasma concentrations and a decreased aortic fatty streak area [16], indicating that it may also modulate lipid metabolism. In addition, several studies have indicated that a cyanidin-rich diet suppresses the high fat diet-induced increase in body weight gain, hyperglycemia, and hyperinsulinemia by inducing adipokine secretion [17] and the upregulation of hormone-sensitive lipase [18]. However, although much data suggest a hypolipidemic effect of cyanidin both *in vitro* and *in vivo*, the direct molecular target remains unknown. We investigated the direct interaction between cyanidin and LBD of PPAR proteins, and examined its hypolipidemic effects in terms of regulation of PPAR target gene expression using selected biomarkers and transcriptome analyses.

2. Materials and methods

2.1. Reagents

Cell culture reagents and supplies were obtained from Hyclone (Logan, UT, USA). Cyanidin (molecular weight is 287.24 g/mol Fig. 1A) was purchased from Extrasynthese (Genay, Cedex, France), fenofibrate, troglitazone, and GW9662 were purchased from Sigma (St. Louis, MO, USA). Lovastatin was purchased from Chungwai Pharmaceutical Company (Gyunggi, Korea). GW0742 was purchased from the Cayman Chemical Company (Ann Arbor, Michigan, USA). Total RNA extraction reagent (RNAiso Plus) and real-time polymerase chain reaction (PCR) premix (SYBR® Premix Ex Taq™) were obtained from Takara (Otsu, Japan). The Oligo $(dT)_{15}$ primer was purchased from Promega (Madison, WI, USA).

2.2. Cell culture and lipid staining

HepG2 and CHO-K1 cells were obtained from the Korean Cell Line Bank (Seoul, Korea), and were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA) and Dulbecco's modified Eagle's medium-F/12 (DMEM-F/12; Hyclone, Logan, UT, USA) medium, respectively, containing 10% heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (PEST; Welgene Inc., Seoul, Korea). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO2.

HepG2 cells were cultured in six-well culture plates for 24 h. Medium was removed and the cells were washed in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate dibasic, 2 mM potassium phosphate monobasic, pH 7.4) twice. Then the cells were treated with free fatty acids (palmitic acid and oleic acid, 400 μM each) with 0.5% bovine serum albumin (BSA, Bovogen Biologicals, Melbourne, Australia) for 24 h, followed by cyanidin (5, 10, 50, or 100 μM) or 10 μM fenofibrate, 1 μM GW0742, and 10 μM troglitazone for a further 24 h, with 1% DMSO as a vehicle. The treated HepG2 cells were stained with oil red O or 5 μL/mL of normal growth medium of DiI dye (Invitrogen, Carlsbad, CA, USA), following the general protocol described previously [19,20]. Cells stained by oil red O was imaged under a Nikon ECLIPSE Ti-S microscope (Nikon, Japan), and cells stained with DiI was imaged under a LSM 5 Exciter Confocal laser scanning microscope (Carl-zeiss, Petaluma, CA, US) at absorption of 549 nM and fluorescence emission maxima of 565 nM.

HepG2 cells were washed twice with 1 mL PBS, treated with 1 mL hexane/isopropanol (2:1) for 30 min at room temperature, and then

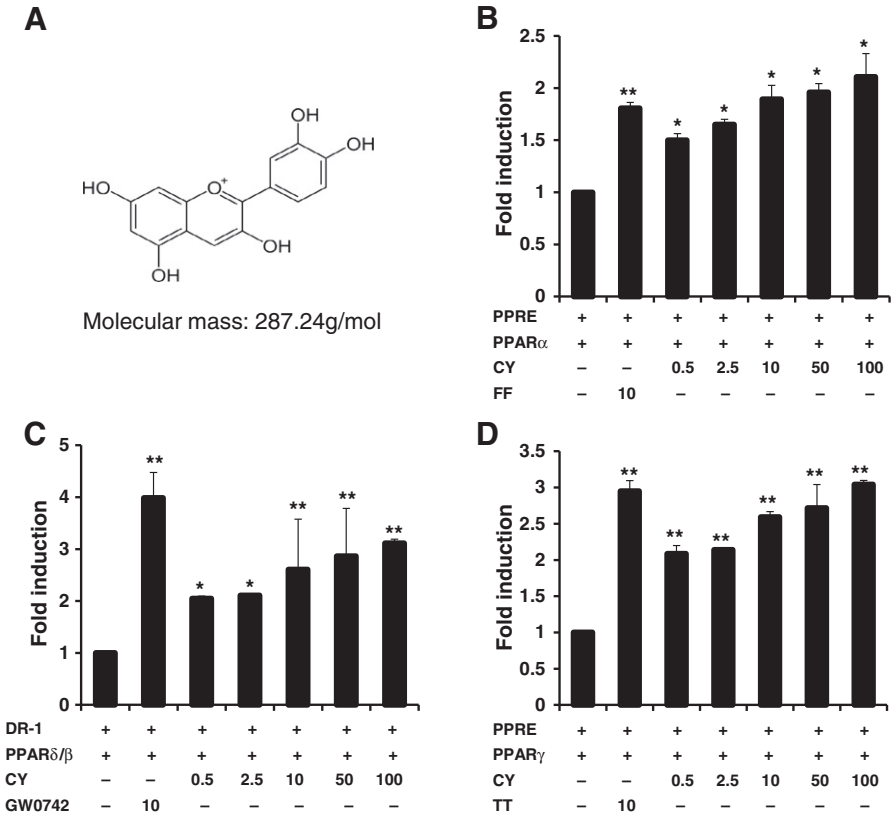

Fig. 1. Cyanidin induces transactivation of PPARα, PPARδ/β, and PPARγ. A. Structure of cyanidin. B–D. Effects of cyanidin on the transactivation of PPARα (B), PPARδ/β (C), and PPARγ (D). Data represent the relative fold increase compared to the non-treated control; all determinations were performed in triplicate. *, $P<0.05$ and **, $P<0.001$ compared to controls.