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Cyanidin, a natural flavonoid, is an agonistic ligand for liver X receptor alpha and beta and reduces cellular lipid accumulation in macrophages and hepatocytes

Yaoyao Jia, Minh Hien Hoang, Hee-Jin Jun, Ji Hae Lee, Sung-Joon Lee *

Department of Biotechnology, The Graduate School of Life Sciences & Biotechnology, Korea University, 5-ga, Anam, Seongbuk, Seoul 136-713, Republic of Korea
Division of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea

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ABSTRACT

Cyanidin, a natural flavonoid abundant in fruits and vegetables, is known to regulate cellular lipid metabolism; however, its underlying mechanism of action and protein targets remain unknown. Here, the ligand binding activity of cyanidin on liver X receptors (LXRs) was investigated utilizing surface plasmon resonance and time-resolved fluorescence energy transfer (TR-FRET) analyses. LXRs are nuclear receptors which function as critical transcription factors in the regulation of cellular lipid and glucose metabolism. This includes the stimulation of high-density-lipoprotein synthesis and activation of reverse cholesterol transport. The present findings show that cyanidin induces the transactivation of LXRs and binds directly to the ligand-binding domain of both LXRα and LXRβ with dissociation constants of 2.2 and 73.2 μM, respectively. Cell-free FRET analysis demonstrated that cyanidin induces the recruitment of co-activator peptide for LXRα and LXRβ with EC_{50} of 3.5 μM and 125.2 μM, respectively. In addition, intracellular cholesterol and triglyceride (TG) concentrations were reduced in macrophages following cyanidin stimulation. In cultured hepatocytes, cyanidin mildly induced SREBP1c gene expression but marginally affected cellular TG concentrations as well as reduced cellular cholesterol accumulations which activated the expression of genes for reverse cholesterol transport. Two cyanidin metabolites, procatechic acid and phloroglucinaldehyde, did not directly bind or activate LXRs. These results demonstrate that cyanidin is a direct ligand for both LXRα and LXRβ, suggesting that cyanidin may operate, at least in part, through modulation of cellular LXR activity.

The liver X receptor (LXR) is a nuclear receptor that induces transcription of target genes in cellular cholesterol, fatty acid, and glucose homeostasis.[1,2] Two LXR isoforms, LXRα and LXRβ, have different tissue distributions with LXRβ being expressed ubiquitously while LXRα expression is restricted to metabolically active tissues such as liver, kidney, intestine, fat tissue, and macrophages with the highest level of expression in the liver.[3]

Known ligands for LXRs, such as oxysterols,[4] glucose,[5] and T0901317,[6] bind to both LXR subtypes and form a heterodimer with the obligate partner 9-*cis* retinoic acid receptor (RXR). This heterodimer binds to the hepatic lipogenesis activating sterol regulatory element binding protein 1c (SREBP1c)[7] as well as the LXR response element (LXRE) in the promoter region of target genes important in cholesterol and glucose metabolism.[8]

Considering the importance of LXRs in the physiology of lipid and cholesterol metabolism, agonists for LXRs have been suggested as a potential therapy for metabolic disorders such as hyperlipidemia and atherosclerosis.[9] LXRs function as cholesterol sensors and regulators of the genes associated with cholesterol absorption, transport, efflux, and excretion and in effect regulate whole body cholesterol homeostasis.[10] Therefore, the activation of LXRs may result in improved reverse cholesterol transport and an increase in circulating high-density-lipoprotein (HDL) levels. Several groups have reported a dose-dependent induction of HDL cholesterol concentrations in C57BL/6 mice following administration of the LXR dual agonist, T0901317.[6] In addition, LXRs act as master regulators of hepatic lipid metabolism. Administration of T0901317 to mice significantly lowers serum and hepatic cholesterol concentrations and inhibits the development of atherosclerosis[11,12] However, synthetic LXR agonists may induce lipogenesis which leads to increased plasma triglyceride (TG) concentrations and hepatic steatosis mainly by inducing SREBP1c in the liver.[6] Accordingly, the development of novel LXR agonists that do not induce hepatic steatosis is of great interest for clinical applications.

* Corresponding author. Tel.: +82 (2) 3290 3029; fax: +82 (2) 3290 3653.
E-mail address: junelee@korea.ac.kr (S.-J. Lee).

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An activity screening experiment using a reporter gene assay identified cyanidin as a potential LXR agonist, which initialized the current study. Cyanidin is an anthocyanin abundant in fruits and vegetables with potent antioxidant and radical-scavenging effects.[13,14] The dietary intake of cyanidin may inhibit the development of dyslipidemia and reduce inflammation.[15] Anthocyanin supplementation including cyanidin in humans decreases the concentrations of low-density lipoprotein (LDL) and increases the concentration of high-density lipoprotein (HDL) cholesterol thus enhancing cellular cholesterol efflux to the serum.[16] The administration of cyanidin-3-*O*-β-glucoside to apoE-deficient mice reduces total serum cholesterol level, increases serum HDL concentration, and ameliorates hypercholesterolemia-induced endothelial dysfunction and atherosclerosis via the ABCG1 pathway.[17] Moreover, the chronic administration of cyanidin-3-glucoside significantly increases HDL-cholesterol whereas significantly declines LDL cholesterol levels without changing total serum cholesterol or TG levels.[18] The administration of red wine phenolic compounds containing cyanidin to hypercholesterolemic Golden Syrian Hamsters significantly reduces plasma cholesterol, TG, and apolipoprotein B concentrations, and decreases the aortic fatty streak area,[18] suggesting that cyanidin may additionally modulate lipid metabolism. Although ample data have shown that cyanidin has hypolipidemic and HDL-enhancing effects, its direct molecular target remains unknown. Here, the direct interaction between cyanidin and the ligand binding domain (LBD) of LXR proteins was investigated in addition to its hypolipidemic effects via regulation of LXR target gene expression.

The transactivation of LXRα and β was induced with stimulation of cyanidin in CHO-K1 cells was assessed with luciferase assay.[19] Cyanidin transactivated LXRα by 32% and 59% at 50 and 100 μM, respectively; while T0901317 (2 μM) induced the promoter activity by 96% compared with controls (Fig. 1B). Cyanidin (100 μM) induced the transactivation activity of LXRβ by 33% whereas T0901317 (2 μM) activated LXRβ promoter by 45% compared with controls (Fig. 1C). The interaction of cyanidin with LXR-LBD was quantified using the surface plasmon resonance (SPR)-BIAcore system[20] and demonstrated that cyanidin directly associated with both LXR subtypes (Fig. 1D and E). The K_D of cyanidin to LXRα and LXRβ was 2.16 and 73.2 μM, respectively (Table 1). The synthetic agonist ligand of the LXRs, T0901317, also directly bound with both LXRα and LXRβ, with a K_D of 92 and 103 nM, respectively. Although the binding affinity of cyanidin to the LXRs was lower compared to T0901317, these results demonstrate that cyanidin directly bound to both subtypes of LXRs with a higher affinity for LXRα than LXRβ.

To further investigate the agonist activity of cyanidin for the two LXR subtypes, LXRα- and LXRβ-LBD proteins were incubated with the co-activator peptide for the corresponding LXR subtype at different concentrations of cyanidin or T0901317 (from 10^{-4}

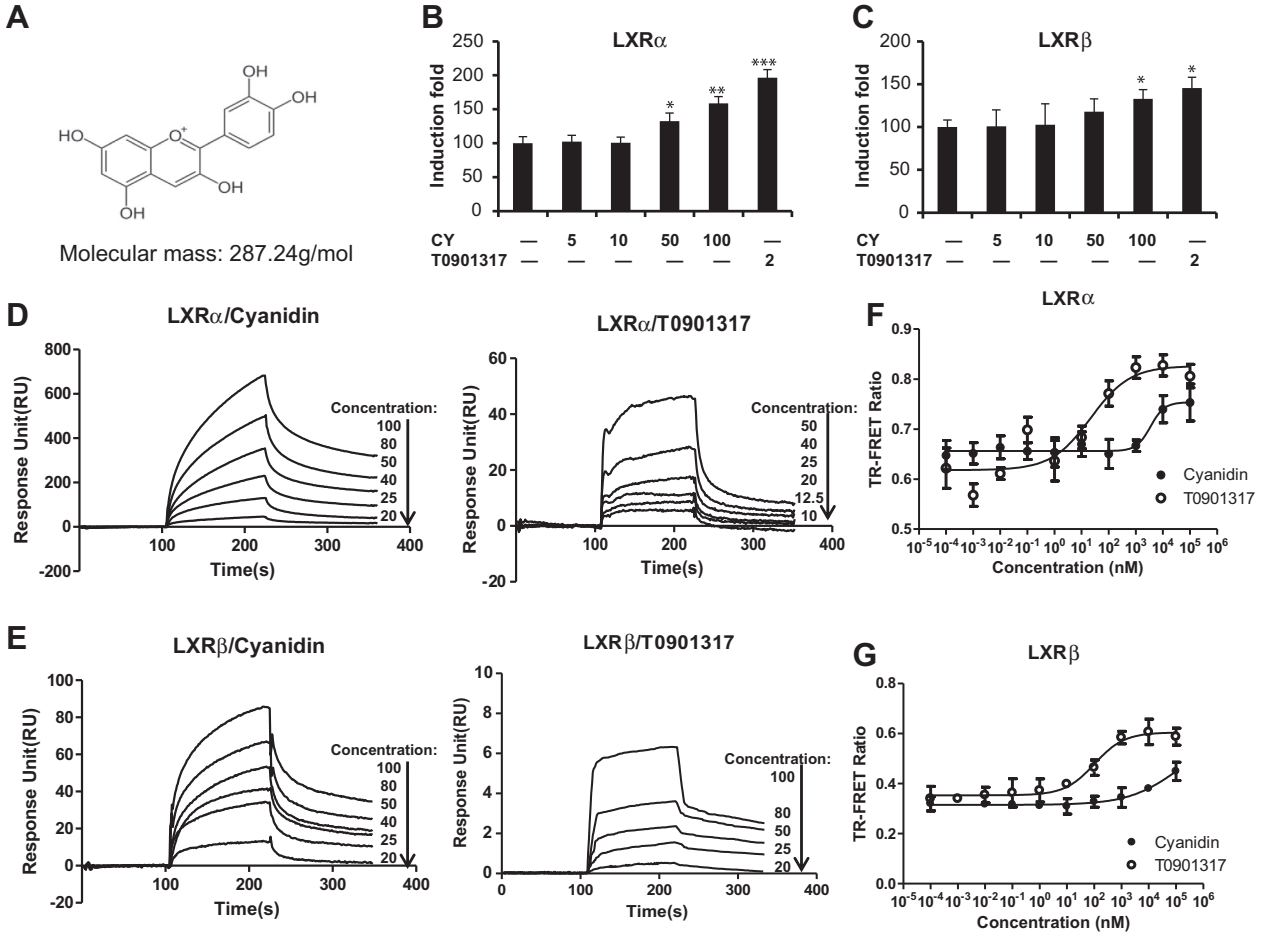

Figure 1. Cyanidin induces transactivation of LXRα and LXRβ, binds directly to LXRα and LXRβ and induces the recruitment of LXR co-activator peptides by activation of LXRα and LXRβ in a TR-FRET assay. (A) Structure of cyanidin. Cyanidin induces transactivation of LXRα (B) and LXRβ (C) measured by luciferase assay. SPR sensorgrams were obtained from Biacore 2000 after injection of a series of concentrations of cyanidin (CY, left panel of B and C), T0901317 (right panel of D and E) over the immobilized hLXRα-LBD or hLXRβ-LBD response. Cyanidin and T0901317 were incubated with LXRα-LBD (F) and LXRβ-LBD (G). $^{*}P$ <0.05, $^{**}P$ <0.001 and $^{***}P$ <0.001 compared to controls. Concentrations are shown in μM.