J Korean Soc Appl Biol Chem (2015) 58(1):13–19
DOI 10.1007/s13765-015-0003-4

Online ISSN 2234-344X
Print ISSN 1738-2203

NOTE

Flavonoids activating adenosine monophosphate-activated protein kinase

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Received: 12 June 2014 / Accepted: 10 July 2014 / Published online: 20 January 2015

Abstract Eighteen flavonoids were tested as adenosine monophosphate-activated protein kinase (AMPK) activators. The AMPK activation induced by kaempferide was 1.75 times better than the value obtained at 100 % AMPK phosphorylation. Western blotting analysis of kaempferide demonstrated that it increased the phosphorylation of AMPK in vitro. In silico docking results carried out to elucidate the binding mode between kaempferide and AMPK showed the docking pose of kaempferide in the binding site of AMPK was similar to that of the substrate contained in the crystallographic structure of AMPK. Flavonoids, including kaempferide, are promising potential AMPK activators.

Keywords Flavonoid · AMPK · Kinase assay · Kaempferide · In silico docking

Introduction

Obesity and Type 2 diabetes receive attention as lifestyle diseases, have gradually increased in occurrence worldwide, lead to a reduction of life expectancy, and increase health problems (Haslam and James 2005). Drugs for diabetes have been researched and developed. Most drugs for Type 2 diabetes are classified as antihyperglycemic agents, which include sulfonylurea derivatives; biguanides, such as metformin, phenformin, buformin, and proguanil; thiazolidinediones; peroxisome proliferator-activated receptor (PPAR-γ) agonists; dipeptidyl peptidase-4 (DPP-4) inhibitors, such as sitagliptin; and human insulin (Zhou et al. 2009). Adenosine monophosphate-activated protein kinase (AMPK) is an enzyme known to control cellular energy, which functions as a cellular energy sensor for metabolic homeostasis, insulin signaling, and metabolic processes (Towler and Hardie 2007). It has been researched as a therapy target for diabetes and obesity since its discovery in 1988 (Fujii et al. 2000; Munday et al. 1988). Mammalian AMPK was purified and sequenced in 1994 (Carattino et al. 2005). Since then, the AMPK activator has been a therapeutic target for Type 2 diabetes. Structurally, AMPK is a heterotrimeric complex and consists of three proteins, as α-, β-, and γ-forms, including isoforms such as α1, 2, β1, 2, γ1, 2, 3 (Carling 2004). Among them, the α-unit plays a catalytic role and the β- and γ-units play regulatory roles (Chen et al. 2012a, b). The complex of each component is strongly correlated with AMPK stability and activity (Stapleton et al. 1996).

AMPK plays a crucial role in associated with metabolic homeostasis, even though its mechanism in this is not completely understood. Among AMPK activities with metabolic effects, there are biological pathways identified such as glucose uptake activation through activation of the glucose transporter 1 (GLUT1) and inhibition of the glucose transporter type 4 (GLUT4) via suppression of Rab-GTPase-activating proteins (TBC1D1); an increase in glycolysis via activation of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2) and PFKFB3; the

Electronic supplementary material The online version of this article (doi:10.1007/s13765-015-0003-4) contains supplementary material, which is available to authorized users.

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activation of fatty acid uptake, even though the intermediary target protein is unknown; regulation of fatty acid synthesis and fatty acid oxidation through Acetyl-CoA carboxylase (ACC1) and ACC2; suppression of cholesterol synthesis by the inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR); and glycogen synthesis inhibition through the repression of glycogen synthase-1 in muscle and glycogen synthase-2 in the liver. These actions support the theory that regulating the effects of increased AMPK expression could be beneficial for treating Type 2 diabetes (Hardie et al. 2012).

Before insulin was used as a drug to treat diabetes, the biguanide metformin was widely used along with alkaloids, such as berberine from plants, which is a known AMPK activator and effective in treating diabetes. Based on these reasons, diverse low-molecular weight compounds were treated as potential drugs for the treatment of diabetes (Bedekar et al. 2010). Metformin remains an effective treatment for diabetes (Lord et al. 2003). It is a derivative of guanidine from natural sources and its structure is similar to galegine (isoprenoid guanidine), which was discovered by Hill for the treatment of diuretic problems (Witters 2001). Guanide derivatives have been used as drugs to control blood sugar since 1920 (Kruger et al. 1959). Among the guanide derivatives, phenformin has been used since the 1960s; however, it was withdrawn due to lactic acidosis (Kwong and Brubacher 1998). Because of this, other types of diabetes drugs, of the thiazolidinedione series, were launched, such as rosiglitazone in 2002, pioglitazone in 2004, and troglitazone in 2006 (Fryer et al. 2002; Saha et al. 2004; LeBrasseur et al. 2006). Among naturally available products, resveratrol, quercetin, and berberine are well-known AMPK inducers. Resveratrol is a polyphenol linked to a long lifespan as well as having a known biological activity. It is contained in wines and there is a lot of information on its role as an AMPK activator; however, its mechanism is not clear (Zang et al. 2006). Quercetin is a flavonoid, which exists in apple and onion. It is also associated with AMPK induction; however, its signal transduction mechanism has not yet been studied (Ahn et al. 2008). Berberine is an isoquinoline alkaloid, which was discovered from traditional plants and is used as a medicine for diabetes (Kong et al. 2004). A final AMPK inducer is α-lipoic acid, which is strongly correlated with AMPK activation and metabolic regulation, even though it is a known cofactor for the mitochondrial respiratory enzyme and shows antioxidant effects (Kim et al. 2004). Several flavonoids, including quercetin and genistein, have shown AMPK activation (Chen et al. 2012a, b). The goal of this research is to elucidate the relationship between the structure of various flavonoids and their activities with respect to AMPK. In addition, one of the test flavonoids showing the strongest AMPK activation, kaempferide, was tested in vitro. Its molecular binding mode with AMPK was examined in silico.

Materials and methods

Eighteen flavonoids, including 13 flavones and 5 isoflavones, were purchased from Indofine Chemical Co. (Hillsborough, NJ, USA). They are listed in Table 1. For the AMPK induction assay, the Z'-LYTE Kinase Assay kit and AMPK (AMPK α1β1γ1, Invitrogen, Carsbad, CA) were used. In order to measure fluorescence resonance energy transfer (FRET) between coumarin and fluorescein, a 96-well plate reader (Molecular Devices, Sunnyvale, CA) was used. All other materials not supplied in the kit, including chemicals (analytical grade) and the black 96-well plates (Corning Incorporated, Tewksbury, MA), were purchased from local suppliers. The procedure for the AMPK induction assay in the 96-well plate was modified based on the supplier's manual. The final concentrations of AMPK and ATP were 0.49 nM and 50 μM, respectively, which achieved approximately 40 % AMPK activation. A 40 μL aliquot was used for the kinase reaction in the 96-well plates, performed at room temperature, and the stock solutions of AMPK, flavonoids, and ATP were prepared as four times concentrated solutions following serial dilution. Quercetin was used as an internal standard for AMPK activation (Ahn et al. 2008). Aliquots of 10 μL of 18 flavonoid stock solutions of 100 μM were added to a mixture of 20 μL kinase (0.98 nM) and Z'-LYTE^{TM} Ser/Thr 23 peptide substrate solution (8 μM), followed by 10 μL of ATP stock solution (200 μM) to start the AMPK activation assay. For the blank test, 4 % dimethyl sulfoxide in kinase buffer solution was used. The final concentration of Z'-LYTE^{TM} peptide substrate solution was 4 μM. In order to mix all solutions thoroughly, the 96-well plate was shaken on plate shaker for 30 s and incubated for 1 h at room temperature. For the fluorescence development reaction to cleave the non-phosphorylated Z'-LYTE peptide substrate, 20 μL of development solution was added to each well. The 96-well plate was shaken for 30 s and then incubated for 1 h. To stop the development reaction, 20 μL of the stop reagent (3 % phosphoric acid solution) was added to each well and the 96-well plate was shaken for 30 s. Immediately, the emission signals were measured on a fluorescence plate reader (excitation: 400 nm, emission 445 nm for coumarin and 520 nm for fluorescein). The ratio of AMPK induction was calculated using the emission ratio, which was the value of the intensity of the coumarin (donor) emission signal at 445 nm divided by that of the fluorescein (acceptor) emission signal at 520 nm. The AMPK induction values of the test flavonoids were determined by

Table 1 The names of the tested flavonoids and their activities

names	Activity
Apigenin (4′,5,7-trihydroxyflavone)	47.4
Biochanin A (5,7-dihydroxy-4′-methoxyisoflavone)	41.5
Daidzein (4′,7-dihydroxyisoflavone)	69.2
Fisetin (3,3′,4′,7-tetrahydroxyflavone)	185.2
Flavone	82.2
Flavonol (7-hydroxyflavone)	167.2
Formononetin (7-hydroxy-4′-methoxyisoflavone)	70.5
Galangin (3,5,7-trihydroxyflavone)	18.1
Genistein (4′,5,7-trihydroxyisoflavone)	52.2
Glycitein (4′,7-dihydroxy-6-methoxyisoflavone)	115.9
Isorhamnetin (3,4′,5,7-tetrahydroxy-3′-methoxyflavone)	167.3
Kaempferide (3,5,7-trihydroxy-4′-methoxyflavone)	175.7
Luteolin (3′,4′,5,7-tetrahydroxyflavone)	100.5
Morin (2′,3,4′,5,7-pentahydroxyflavone)	167.6
Myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone)	110.6
Quercetin (3,3′,4′,5,7-pentaflavone)	151.8
Scutellarein (4′,5,6,7-tetrahydroxyflavone)	145.2
Sinensetin (3′,4′,5,6,7-pentamethoxyflavone)	16.8

comparison with the ratio of AMPK induction obtained at a 100 % phosphorylation (100) (Table 1).

3T3L1 preadipocytes were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS; Hyclone, Logan, UT, USA). The 3T3L1 cells were treated with 20 μM quercetin or 20 μM kaempferide for different time periods, harvested and lysed in a buffer containing 20 mM HEPES (pH 7.2), 1 % Triton X-100, 10 % glycerol, 150 mM NaCl, 10 μg/mL leupeptin, and 1 mM phenylmethylsulfonyl fluoride (PMSF). Electrophoresis and immunoblotting were performed as previously described (Shin et al. 2014). Anti-phospho-AMPKα (Thr172) was purchased from Cell Signaling Technology (Beverly, MA, USA). Signals were developed using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA).

To elucidate the molecular binding mode between kaempferide and AMPK, in silico docking was performed. The three-dimensional (3D) structure of AMPK used was the crystallographic structure of human AMPK α1β2γ1 deposited in the protein data bank (PDB) as 2ya3.pdb (Xiao et al. 2011). The docking experiments were carried out on an Intel Core 2 Quad Q6600 (2.4 GHz) Linux PC with Sybyl 7.3 (Tripos) (Shin et al. 2013). To identify the binding site between kaempferide and AMPK, 2ya3.pdb was analyzed using the LigPlot program (Kramer et al. 1999). The 3D images were generated using the PyMOL software (The PyMOL Molecular Graphics System, Version 1.0r1, Schrödinger, LLC, Portland, OR, USA).

Results and discussion

Even though 18 compounds are not enough to quantitatively determine structural properties and biological activity relationships (SAR), they are sufficient to qualitatively elucidate SAR. All the flavonoids tested, except flavone and sinensetin, contain a 7-hydroxyl group, so it can be deduced that 7-OH is not vital in determining AMPK activation ability, but 7-OH increases activity if you compare 7-hydroxyflavone with flavone. Unlike the 7-hydroxyl group, the 3-hydroxyl group contributes to activity comparing quercetin with luteolin. The 2′-Methoxy group contributes to activity more than the 3′-OMe in morin and isorhamnetin. Two hydroxyl groups at the ortho position favor AMPK activation in fisetin, quercetin, myricetin, and luteolin. However, two methoxy groups at the ortho position do not favor AMPK activation. Three sequencing hydroxyl groups at the ortho and meta positions do not contribute to activity in myricetin and quercetin. Flavones and isoflavones are distinguished based on the attachment of the benzene ring to the 4*H*-chromen-4-one moiety of flavonoids, where the former is at 2-phenyl-4*H*-chromen-4-one, and the latter is at 3-phenyl-4*H*-chromen-4-one. The abilities of isoflavones to activate AMPK are considered better than those of flavones because the activity of genistein is better than that of apigenin. Even though a 4′-methoxy group is not important for determining AMPK activation ability, methoxy groups substituted at the A-ring, named for the benzene ring of the 4*H*-chromen-4-one moiety (Supplementary Fig. 1), decrease activity in sinensetin. Further experiments for quantitative SAR are required (Shin et al. 2011).

Fisetin, showing the greatest ability to activate AMPK, is a well-documented AMPK activator (Shin et al. 2011). However, the AMPK activation ability of the second ranked flavonoid among the test compounds, kaempferide, has not been reported. It has been demonstrated that AMPK becomes activated when the threonine-172 residue is phosphorylated (Hawley et al. 1996). To determine whether kaempferide activates AMPK, a phospho-specific immunoblot analysis was performed. Quercetin was used as a positive reference compound (Shen et al. 2012; Lu et al. 2010). Treatment with 20 μM quercetin increased the level of phosphorylated AMPK (threonine-172) within 30 min, which then slowly decreased (Fig. 1a). Similarly, kaempferide induced the phosphorylation of AMPK within 30 min, suggesting that kaempferide rapidly activates the catalytic activity of AMPK (Fig. 1b).

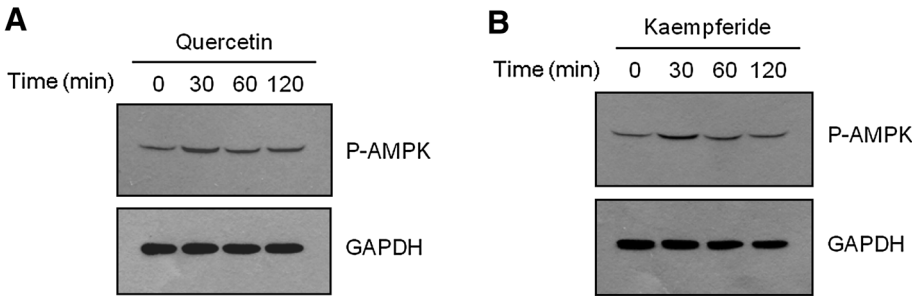

Fig. 1 3T3L1 preadipocytes were treated with 20 μM quercetin (**a**) or 20 μM kaempferide (**b**) for the indicated times. Whole-cell lysates were prepared and immunoblot analysis was performed using anti-phospho adenosine monophosphate-activated protein kinase (AMPK) (Thr172). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control

Kaempferide, 3,5,7-trihydroxy-4′-methoxyflavone is found in the plants such as *Kaempferia galanga* (aromatic ginger) and *Alpinia oxyphylla* (Docherty and Bexis 2013). It increases the melanin content in B16F10 melanoma cells (Horibe et al. 2013). Even though its activation of AMPK has not been reported, it is known to increase glucose uptake activity (Ueda et al. 2013); thus, supporting the current result that kaempferide activates the phosphorylation of AMPK. To elucidate the molecular binding mode between AMPK and kaempferide, in silico docking was carried out. AMPK consists of three subunits, α, β, and γ, which are composed of two, two, and three isoforms, respectively, such that 12 possible AMPK heterotimeric isomers can exist (Kahn et al. 2005). The α subunit

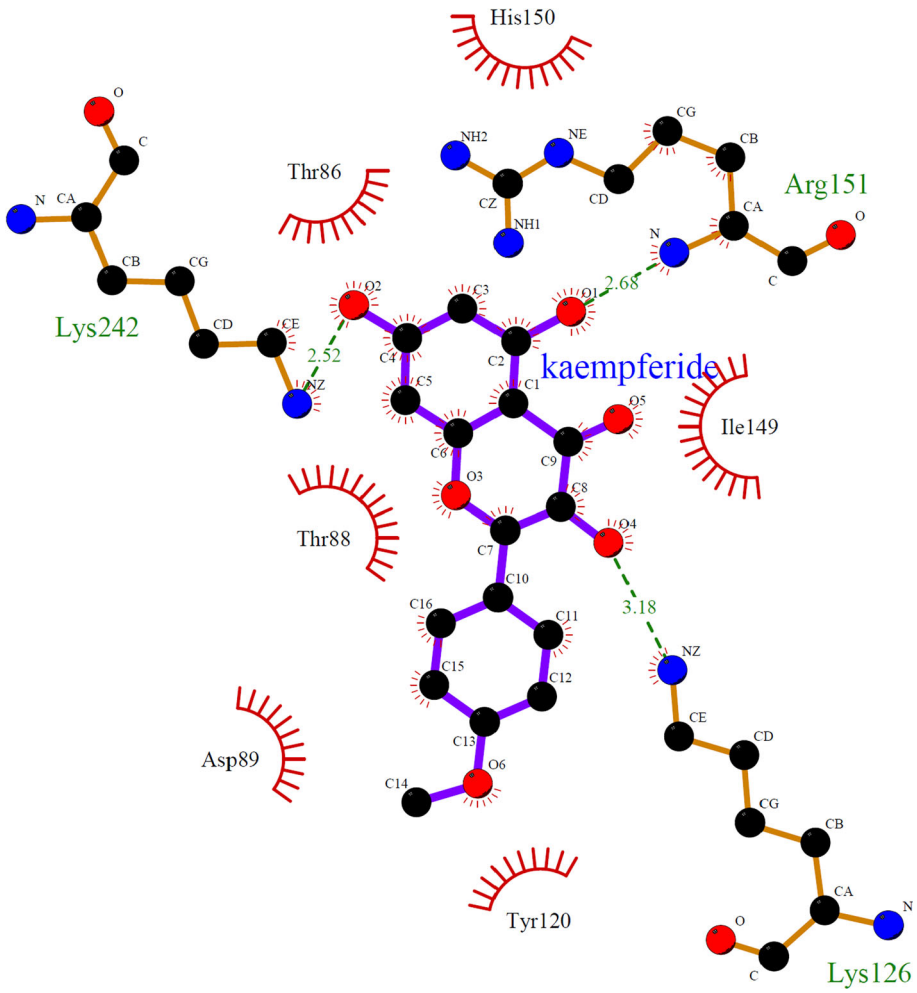

Fig. 2 The binding site of kaempferide contained in adenosine monophosphate-activated protein kinase (AMPK) analyzed by the LigPlot program where half red circles indicate the residues participating in hydrophobic interactions and the dotted lines show hydrogen bonds

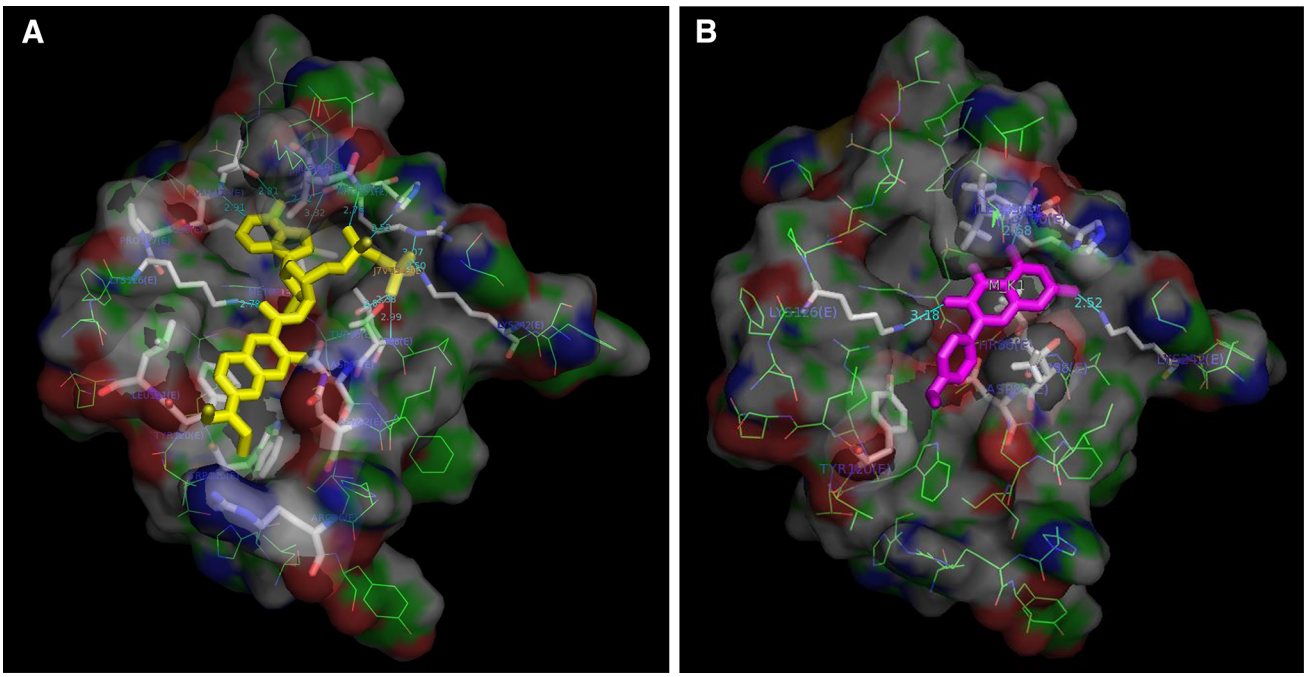

Fig. 3 **a** The 3D image of the binding site of the 7-diethylaminocoumarin-3-carbonylamino-ADP-adenosine monophosphate-activated protein kinase (AMPK) complex generated using the PyMOL program and **b** that of the kaempferide-AMPK complex. 7-Diethylaminocoumarin-3-carbonylamino-ADP and kaempferide are colored in *yellow* and *magenta*, respectively

contains a β subunit-binding domain in the C-terminal region, and the β subunit has an α and a γ subunit-binding domain in the C-terminal region. The γ subunit includes a β subunit-binding domain in the N-terminal region (Hardie et al. 2012). Many crystallographic structures of AMPK are found in the PDB. If monomer structures are excluded, the heterotrimeric structures of α2β1γ1 and α1β2γ1 remain. The β1 and β2 isoforms have common features in their C-terminal region, and the substrate binds to the α or γ subunits, therefore, the heterotrimeric structure of α1β2γ1 was selected for in silico docking experiments. In addition, the 3D structures of AMPK with coumarin and staurosporine as a substrate were found; because flavonoids are more similar to coumarin than staurosporine, the α1β2γ1 and coumarin complex (2ya3.pdb) was used. The α1, β2, and γ1 subunits consist of 559, 272, and 330 residues, respectively. In 2ya3.pdb, the 3D crystallographic structures of C-terminal 149 residues of Rattus norvegicus α1, C-terminal 83 residues of Homo sapiens β2, and 299 residues of the whole region of Rattus norvegicus γ1 were determined (Xiao et al. 2011). Two 7-diethylaminocoumarin-3-carbonylamino-ADPs (DCA) bind to the γ1 subunit where one substrate resides near the N-terminal region and another resides toward the C-terminal region. AMPK is activated allosterically (Yeh et al. 1980). Therefore, the binding pocket of the substrate residing near the N-terminal region was analyzed using the LigPlot program (Kramer et al. 1999). The residues, Let84, Asp89, Asn92, Arg96, Trp116, Tyr120, Leu121, Pro127, Leu128, Ile149, and Pro153 participated in hydrophobic interactions and Thr86, Thr88, Lys126, Val129, His150, Arg151, and Lys242 formed hydrogen bonds (H-bonds) (Supplementary Fig. 2). The apo-AMPK protein was prepared by deleting the ligand DCA using the Sybyl program. The 3D structure of kaempferide was determined based on the 3D structure of kaempferol contained in the crystallographic complex of quercetin 2,3-dioxygenase using molecular modeling (Steiner et al. 2002). Because the flexible docking method was used, the docking process was iterated 30 times (Shin et al. 2013). As a result, 30 complexes between apo-AMPK and kaempferide were generated. All kaempferides bound to AMPK were docked well (Supplementary Fig. 3). Their binding energy ranged from −22.55 to −11.41 kcal/mol. Of the 30 complexes, complex 12 was selected because its docking pose was similar to the pose of the ligand DCA of 2ya3.pdb. The binding energy of complex 12 was −13.46 kcal/mol. The residues residing in the binding site of kaempferide were Thr86, Thr88, Asp89, Tyr120, Ile149, and His150 (hydrophobic interactions) and Lys126, Arg151, and Lys242 (H-bonds) (Fig. 2). All residues were also found in the binding site of DCA. In addition, three residues forming H-bonds with kaempferide were contained in seven residues forming H-bonds with DCA. The 3D images of the binding site residing in DCA (Fig. 3a) and kaempferide (Fig. 3b) were generated by PyMol. In the kaempferide-AMPK complex, the distances between the

oxygens of 3-OH, 5-OH, and 7-OH, and the nitrogens of the amine group of Lys126, the amide bond of Arg151, and the amine group of Lys242 were 3.18, 2.68, and 2.52Å, respectively. In the DCA-AMPK complex, the distances between the oxygen of the amide bond and nitrogen of the amine group of Lys126, the nitrogen of purine and oxygen of the amide bond of Arg151, and the oxygen of the phosphate group and nitrogen of the amine group of Lys242 were 2.78, 3.32, and 2.50Å, respectively. Therefore, comparing the 3D images, their docking poses were similar to each other but the binding site of DCA was larger than that of kaempferide, because of its small size.

Since one of the flavonoids, quercetin, is an AMPK activator, 18 flavonoids including 13 flavones and 5 isoflavones were tested to determine whether they could activate the phosphorylation of AMPK. The AMPK activation induced by kaempferide was 1.75 times better than the value obtained at 100 % AMPK phosphorylation. Western blotting analysis of kaempferide demonstrated that it increased the phosphorylation of AMPK in vitro. In silico docking results carried out to elucidate the binding mode between kaempferide and AMPK at the molecular level showed that the docking pose of kaempferide in the binding site of AMPK is similar to that of the substrate contained in the crystallographic structure of AMPK. In conclusion, flavonoids including kaempferide could be promising potential AMPK activators.

Acknowledgments This research was supported by a grant from the Korean Health Technology R&D project, Ministry of Health & Welfare, Republic of Korea (A111698). SY Shin was supported by the KU Research Professor Program of Konkuk University.

References

Ahn J, Lee H, Kim S, Park J, Ha T (2008) The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem Biophys Res Commun 373:545–549

Bedekar A, Shah K, Koffas M (2010) Natural products for type II diabetes treatment. Adv Appl Microbiol 71:21–73

Carattino M, Edinger R, Grieser H, Wise R, Neumann D, Schlattner U, Johnson J, Kleyman T, Hallows K (2005) Epithelial sodium channel inhibition by AMP-activated protein kinase in oocytes and polarized renal epithelial cells. J Biol Chem 280: 17608–17616

Carling D (2004) The AMP-activated protein kinase cascade–a unifying system for energy control. Trends Biochem Sci 29: 18–24

Chen D, Pamu S, Cui Q, Chan TH, Dou QP (2012a) Novel epigallocatechin gallate (EGCG) analogs activate AMP-activated protein kinase pathway and target cancer stem cells. Bioorg Med Chem 20:3031–3037

Chen L, Wang J, Zhang Y, Yan S, Neumann D, Schlattner U, Wang Z, Wu Z (2012b) AMP-activated protein kinase undergoes nucleotide-dependent conformational changes. Nat Struct Mol Biol 19:716–718

Docherty J, Bexis S (2013) Influence of ketanserin on the effects of methylenedioxymethamphetamine on body temperature in the mouse. Auton Autacoid Pharmacol 33:35–41

Fryer L, Parbu-Patel A, Carling D (2002) The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 277:25226–25232

Fujii N, Hayashi T, Hirshman M, Smith J, Habinowski S, Kaijser L, Mu J, Ljungqvist O, Birnbaum M, Witters L, Thorell A, Goodyear L (2000) Exercise induces isoform-specific increase in 5′AMP-activated protein kinase activity in human skeletal muscle. Biochem Biophys Res Commun 273:1150–1155

Hardie D, Ross F, Hawley S (2012) AMP-activated protein kinase: a target for drugs both ancient and modern. Chem Biol 19: 1222–12236

Haslam D, James W (2005) Obesity. Lancet 366:1197–1209

Hawley S, Davison M, Woods A, Davies S, Beri R, Carling D, Hardie D (1996) Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. J Biol Chem 271:27879–27887

Horibe I, Satoh Y, Shiota Y, Kumagai A, Horike N, Takemori H, Uesato S, Sugie S, Obata K, Kawahara H, Nagaoka Y (2013) Induction of melanogenesis by 4′-*O*-methylated flavonoids in B16F10 melanoma cells. J Nat Med 67:705–710

Kahn B, Alquier T, Carling D, Hardie D (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1:15–25

Kim M, Park J, Namkoong C, Jang P, Ryu J, Song H, Yun J, Namgoong I, Ha J, Park I, Lee I, Viollet B, Youn J, Lee H, Lee K (2004) Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. Nat Med 10:727–733

Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, Wang Y, Wang Z, Si S, Pan H, Wang S, Wu J, Wang Y, Li Z, Liu J, Jiang J (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 10: 1344–1351

Kramer B, Rarey M, Lengauer T (1999) Evaluation of the FLEXX incremental construction algorithm for protein-ligand docking. Proteins 37:228–241

Kruger F, Skillman T, Hamwi G, Grubbs R, Nicholas D (1959) The mechanism of action of hypoglycemic guanidine derivative. Nutr Rev 17:243–244

Kwong S, Brubacher J (1998) Phenformin and lactic acidosis: a case report and review. J Emerg Med 16:881–886

LeBrasseur N, Kelly M, Tsao T, Farmer S, Saha A, Ruderman N, Tomas E (2006) Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. Am J Physiol Endocrinol Metab 291:E175–E181

Lord J, Flight I, Norman R (2003) Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 327:951–953

Lu J, Wu D, Zheng Y, Hu B, Zhang Z, Shan Q, Zheng Z, Liu C, Wang Y (2010) Quercetin activates AMP-activated protein kinase by reducing PP2C expression protecting old mouse brain against high cholesterol-induced neurotoxicity. J Pathol 222:199–212

Munday M, Campbell D, Carling D, Hardie D (1988) Identification by amino acid sequencing of three major regulatory phosphorylation sites on rat actyl-CoA carboxylase. Eur J Biochem 175:331–338

Saha A, Avilucea P, Ye J, Assifi M, Kraegen E, Ruderman N (2004) Pioglitazone treatment activates AMP-activated protein kinase in rat liver and adipose tissue in vivo. Biochem Biophys Res Commun 314:580–585

Shen Y, Croft K, Hodgson J, Kyle R, Lee I, Wang Y, Stocker R, Ward N (2012) Quercetin and its metabolites improve vessel function

by inducing eNOS activity via phosphorylation of AMPK. Biochem Pharmacol 84:1036–1044

Shin S, Woo Y, Hyun J, Yong Y, Koh D, Lee Y, Lim Y (2011) Relationship between the structures of flavonoids and their NF-κB-dependent transcriptional activities. Bioorg Med Chem Lett 21:6036-2641

Shin S, Yoon H, Hwang D, Ahn S, Kim D, Koh D, Lee Y, Lim Y (2013) Benzochalcones bearing pyrazoline moieties show anti-colorectal cancer activities and selective inhibitory effects on aurora kinases. Bioorg Med Chem 21:7018–7124

Shin S, Jung H, Ahn S, Hwang D, Yoon H, Hyun J, Yong Y, Cho H, Koh D, Lee Y, Lim Y (2014) Polyphenols bearing cinnamaldehyde scaffold showing cell growth inhibitory effects on the cisplatin-resistant A2780/Cis ovarian cancer cells. Bioorg Med Chem 22:1809–1820

Stapleton D, Mitchelhill K, Gao G, Widmer J, Michell B, Teh T, House C, Fernandez C, Cox T, Witters L, Kemp B (1996) Mammalian AMP-activated protein kinase subfamily. J Biol Chem 271:611–614

Steiner R, Kalk K, Dijkstra B (2002) Anaerobic enzyme substrate structures provide insight into the reaction mechanism of the copper-dependent quercetin 2,3-dioxygenase. Proc Natl Acad Sci 99:16625–16630

Towler M, Hardie D (2007) AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 100:328–341

Ueda M, Hayashibara K, Ashida H (2013) Propolis extract promotes translocation of glucose transporter 4 and glucose uptake through both PI3K- and AMPK-dependent pathways in skeletal muscle. BioFactors 39:457–466

Witters L (2001) The blooming of the French lilac. J Clin Invest 108:1105–1107

Xiao B, Sanders M, Underwood E, Heath R, Mayer F, Carmena D, Jing C, Walker P, Eccleston J, Haire L, Saiu P, Howell S, Aasland R, Martin S, Carling D, Gamblin S (2011) Structure of mammalian AMPK and its regulation by ADP. Nature 472:230–233

Yeh L, Lee K, Kim K (1980) Regulation of rat liver acetyl-CoA carboxylase. Regulation of phosphorylation and inactivation of acetyl-CoA carboxylase by the adenylate energy charge. J Biol Chem 255:2308–2314

Zang M, Xu S, Maitland-Toolan K, Zuccollo A, Hou X, Jiang B, Wierzbicki M, Verbeuren T, Cohen R (2006) Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. Diabetes 55:2180–2191

Zhou G, Sebhat I, Zhang B (2009) AMPK activators–potential therapeutics for metabolic and other diseases. Acta Physiol 196:175–190