J. Appl. Biol. Chem. **51**(6), 253-261 (2008)

Review

Isoflavone Daidzein: Chemistry and Bacterial Metabolism

Mihyang Kim[1], Jaehong Han[2], and Soo-Un Kim[1,*]

[1]*Program in Applied Biological Chemistry, Department of Agricultural Biotechnology and Research Institute for Agricultural Sciences, Seoul National University, Seoul 151-921, Republic of Korea*

[2]*Metalloenzyme Research Group and Department of Biotechnology, Chung-Ang University, Anseong 456-756, Republic of Korea*

Received November 14, 2008; Accepted December 16, 2008

Isoflavone daidzein is a phytoestrogen widely distributed in Leguminosae and is especially rich in the soybean. The C6-C3 (rings B and C) unit of isoflavones is derived from the phenylpropanoid pathway and the remaining C6 (ring A) unit is from the polyketide pathway. This unique carbon skeleton is the result of isomerization of the flavone catalyzed by the isoflavone synthase, a cytochrome P450 enzyme. The isoflavones daidzein and genistein are present in the plant mostly in the glucosylated forms. However, in the human intestine, the glycosidic linkage is broken, and the free form is uptaked into blood stream. The free form is further metabolized into various reduction products to end up at the equol, which is known to have the most potent estrogenic effect among the metabolites. Several human intestinal bacteria that can convert daidzein into equol have been described, and the study into the chemistry and biochemistry of the daizein reduction would be rewarding to the improvement of the human health.

Key words: *daizein, equol, genistein, intestinal microflora, isoflavonoids*

Nomenclature and occurences of isoflavone

The term "flavonoids" is generally used to describe a class of natural products that is comprised of a C_6-C_3-C_6 carbon framework, or phenylbenzopyran (or chroman) functionality [Marais *et al.*, 2006]. Depending on the position of the linkage of the aromatic ring to the benzopyrano moiety, they can be classified into three subclasses; flavonoids, isoflavonoids, and neoflavonoids (Fig. 1).

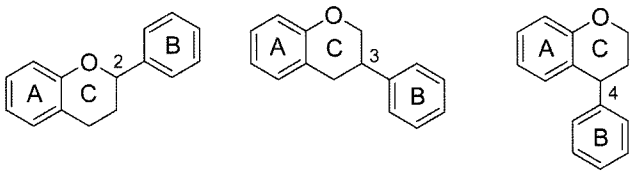

Fig. 1. Basic structures of flavonoids, isoflavonoids, and neoflavonoids.

In this C_6-C_3-C_6 carbon framework, the two rings of chroman moiety are labeled as A and C rings, and the attached phenyl group as B ring. Isoflavonoid, a distinctive subclass of the flavonoids, differ from the flavonoids by the position of ring B on ring C. Whereas the B ring is located at C-2 in the flavonoids, in the isoflavonoids it is found at C-3. The numbering system of the isoflavone is shown in Fig. 2. Isoflavonoids are further classified into isoflavone, isoflavanone, and isoflavanol according to their functionality on C1 through C3. Isoflavanone and isoflavonol have two and three chiral centers, respectively. Recently, the correlation between the absolute configuration and the chiroptical property, the Cotton effect, has been elucidated [Won *et al.*, 2008]. This finding will facilitate the determination of the absolute configuration of various isoflavanol metabolites in humans.

Plants are capable of accumulating large variety of low molecular-weight organic compounds, which are collectively termed as the secondary metabolites. These metabolites play important roles in the plant-environments interactions, as well as in the human nutrition and the medicine. Isoflavonoids are important secondary metabolites in Leguminosae that is capable of harboring symbiotic bacteria internalized within the root nodules, which play a

*Corresponding author
Phone: +82-2-880-4642; Fax: +82-2-873-3112
E-mail: soounkim@plaza.snu.ac.kr

doi:10.3839/jabc.2008.040

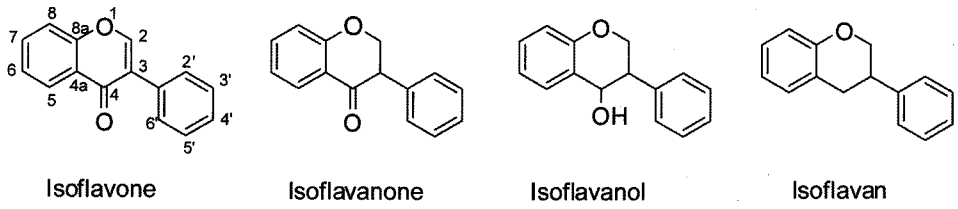

Fig. 2. Structures, names, and numbering system of the simple isoflavonoids.

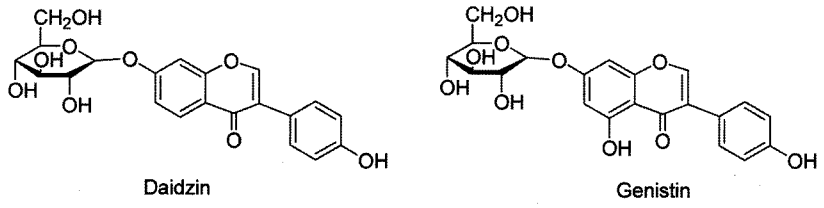

Fig. 3. Structures of daidzin and genistin.

protective role against microbial infection [Blount *et al.*, 1992].

The isoflavones are the largest group of isoflavonoids, and the legumes are the most important sources of isoflavones. The concentration of isoflavones in the soybeans is much higher than those in other legumes. Therefore, soy and soy-based foods are regarded as the main sources of isoflavones in the human diet [Mazur and Adlercruetz 1998; Liggins *et al.*, 2000]. The most abundant isoflavones are daidzein (7,4'-dihydroxyisoflavone) and genistein (5,7,4'-trihydroxyisoflavone), which together comprise 0.1-0.3 mg/g soybean [Wang and Murphy, 1994]. In plants the isoflavonoids mostly occur as sugar conjugates, frequently with glucose at the 7-*O*-position. The glucoside form of daidzein and genistein are called daidzin and genistin, respectively (Fig. 3). They are abundant in the soybean products, because they are heat stable [Setchell 1998].

Biosynthesis of isoflavone daidzein

Isoflavonoids are synthesized by a branch of the phenylpropanoid pathway in plants. The flavonoids, lignin, and anthocyanin pigments also share the phenylpropanoid pathway [Winkel-Shirley, 2001]. Ring B and part of the heterocyclic ring C are formed from 4-coumaric acid co-enzyme A (CoA) ester via shikimate pathway, starting from carbohydrate (Fig. 4). Ring A is formed via the polyketide pathway from three units of malonyl CoA, derived from acetyl CoA and carbon dioxide. Chalcones are synthesized via the addition of three malonyl groups into coumarate by chalcone synthase (CHS), which catalyzes the stepwise condensation of these precursors to a C15 intermediate. Chalcone isomerase (CHI) catalyzes the stereospecific cyclization of chalcone to form flavanone (Fig. 4).

The committed step for the isoflavonoid biosynthesis is a unique aryl migration reaction catalyzed by a cytochrome P450 enzyme CYP93C, isoflavone synthase (IFS). The reaction is initiated by the abstraction of a hydrogen to form a radical at C-3 of the flavanone. The next step, the migration of ring B from C-2 to C-3 and subsequent hydroxylation of the resulting C-2 radical, completes the isomerization. Isoflavone synthase catalysis is regioselective, and only (2*S*)-flavanones are stereospecifically transformed into (2*R*,3*S*)-hydroxyisoflavanone. The product of the IFS reaction is the 2-hydroxyisoflavanone, which can be dehydrated into isoflavones either spontaneously or through the catalysis by 2-hydroxyisoflavanon dehydratase [Akashi *et al.*, 1995; 2005].

Genistein is the end product of the isoflavone synthase and 2-hydroxyisoflavanone dehydratase reactions when naringenin is used as the substrate (Fig. 4). Genistein biosynthesis shares the naringenin intermediate with the flavonoid/anthocyanin branch of the phenylpropanoid pathway, but is also the building block of many, structurally more complicated isoflavonoids compounds.

Because of the structural complexity of the secondary metabolites, their chemical synthesis is often difficult and expensive, partly due to the very low yields. Therefore, manipulation of the biosynthetic pathways to attain the accumulation of the isoflavonoid in plants through the expression of endogenous genes or by introducing foreign genes has been considered. Recent studies on tobacco, Arabidopsis, and maize demonstrated that isoflavonoids can be accumulated to a high level in the non-legume transgenic plants when soybean isoflavone synthase gene is incorporated through metabolic engineering [Yu *et al.*, 2001; Liu *et al.*, 2002; Yu *et al.*, 2003; Tian and Dixon 2006].

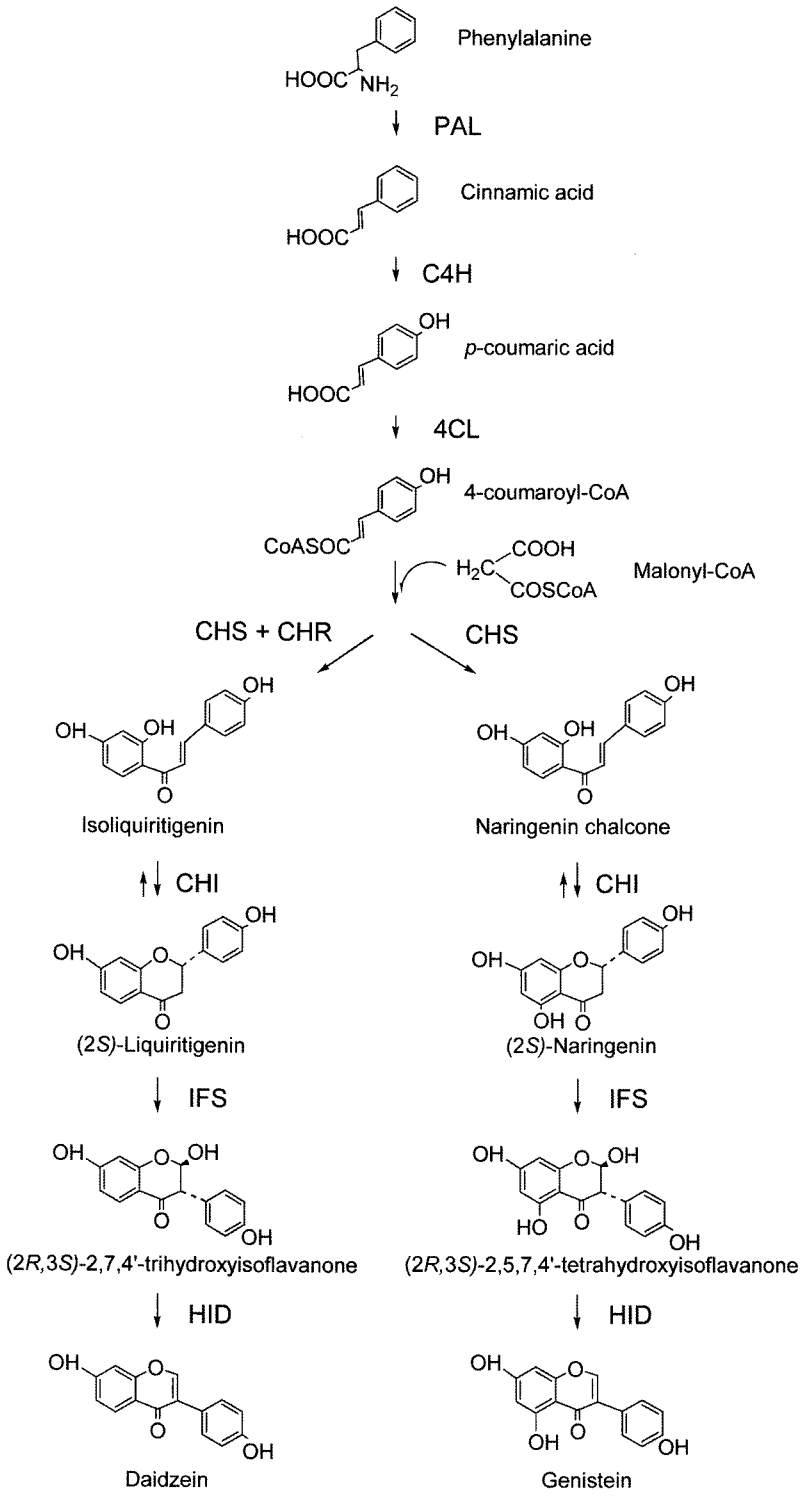

Fig. 4. Modified pathway of isoflavone biosynthesis in legumes [Tian and Dixon, 2006]. PAL, L-phenylalanine ammonia-lyase; C4H, cinnamate-4-hydroxylse; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; CHR, chalcone reductase; CHI, chalcone isomerase; IFS, isoflavone synthase; HID, 2-hydroxylisoflavanone dehydratase.

Bioactivities of daidzein and its derivatives

Isoflavonoids were initially recognized for their roles in plant disease resistance and induction of nodulation [Lee *et al.* 1991]. However, in recent years they received much attention due to their estrogenic, antioxidant, and anticancer activities on humans [Cornwell *et al.*, 2004; Dixon, 2004].

The incidences of the hormone-related disease, such as breast and prostate cancers, in the Asians with a high

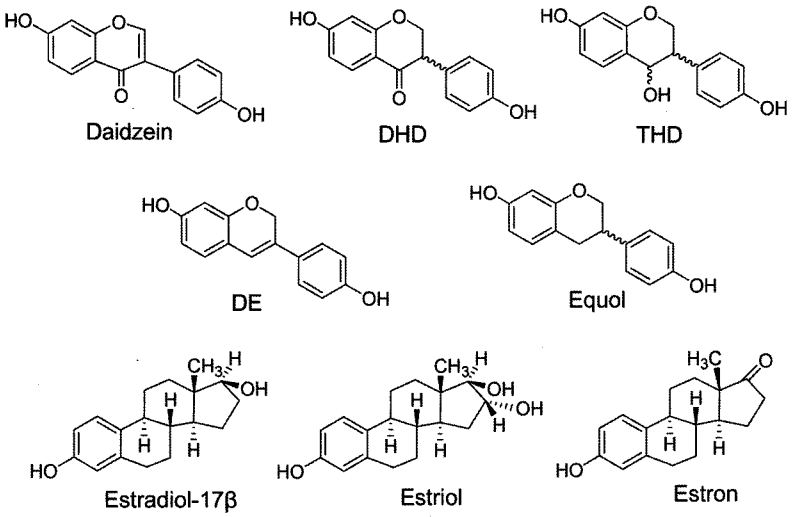

Fig. 5 . Chemical structures of daidzein, its metabolites, and three human estrogens.

isoflavone intake are significantly lower than those of the Western counterparts. For examples, the average intake of isoflavones among the Japanese adults was estimated as 30-50 mg/day in the forms of miso, tofu, and soy sauce [Wakai *et al.*, 1999], and the Chinese adults also consume similar amounts of isoflavones (~40 mg/day) [Chen *et al.*, 1999]. The Korean population was estimated to take up 15 mg/day of isoflavonoids [Kim and Kwon, 2001]. In contrast, the intake of isoflavones is very low in the Western population, typically less than 5 mg/day isoflavones. Urinary excretion of isoflavones and their metaboltes were shown to be 13-30 times lower in USA and England than in Japan [Herman *et al.*, 1995; Mazur and Adlercreutz, 2000].

Estrogens are strongly bound to the serum proteins, albumin, and sex hormone-binding globulin, whereas phytoestrogens are weakly bound to these serum proteins. For example, equol shows 10-fold less affinity than estradiol, thus enabling a greater proportion of equol to occupy the estrogen receptor. This could explain the effectiveness of isoflavones [Setchell and Cassidy, 1999; Dixon and Sumner, 2003]. The molecular structures of daidzein, its metabolites, and three human estrogens are given in Fig. 5.

Daidzein has been shown to be less active than genistein in the estrogenic activity [Verma and Goldin, 1998]. However, daidzein, through the reductive metabolism, is converted into its metabolites, including DHD (dihydrodaidzein), *cis*- and *trans*-THDs (tetrahydrodaidzeins), *O*-desmethylangolensin, DE (dehydroequol), and equol, by the intestinal microflora after ingestion [Kelly *et al.*, 1995]. Lehmann *et al.* [2005] reported the estrogenic and genotoxic potentials of equol. Interestingly, among the metabolites of daidzein, (3*S*)-equol has about 100 times higher estrogenic activity than the daidzein itself [Hwang *et al.*, 2006].

Daidzein was shown to inhibit the growth of human prostate and the estrogen-sensitive breast cancer cells MCF-7 [Adlercreutz, 2003; Cross *et al.* 2004; Holzbereierlein *et al.* 2005; Matsumura *et al.*, 2005]. Choi and Kim [2008] also reported that daidzein significantly inhibited the cell proliferation dose- and time-dependently via the cell cycle arrest in G1 and G2/M phases. Wähälä *et al.* [1997] showed that THD was more active than daidzein, and the activity was comparable to that of genistein against the prostate cancer cells. On the other hand, 3'-OH-daidzein and 6-OH-daidzein, the oxidized forms of the daidzein metabolite, showed markedly lower activities.

Dietary daidzein may lower the cholesterol level by increasing the receptor activity of low-density lipoprotein, and this reduction in the cholesterol level may offer some protection against atherosclerosis [Kirk *et al.*, 1998]. Jiang *et al.* [2003] showed that DHD could decrease both the total cholesterol and the proatherogenic non-HDL cholesterol levels. The cardiovascular protective activities of *cis*-THD, *trans*-THD, DE, and DHD have been elucidated [Chin-Dusting *et al.* 2001; Chin-Dusting *et al.* 2004; Kanellakis *et al.* 2004; Ling *et al.* 2004; Nestel *et al.* 2007].

Daidzein metabolism in human

Intestinal microflora plays a key role in the metabolism and the bioavailability of daidzein [Setchell *et al.* 1984]. Daidzein metabolites occur in blood plasma, urine, and feces of humans as a result of the biotransformation by the intestinal microorganisms. The absorption of daidzein appears to be dependent on the specific enzyme that is

capable of hydrolyzing the sugar moiety. This enzyme is found in the small intestinal brush border and enterocytes. Daidzein may be directly absorbed or further metabolized by the microflora into its metabolites, including dihydrodaidzein (DHD) and equol before absorption. The presence of this reductive metabolism, however, appears to vary among individuals.

Like endogenous estrogen, daidzein undergoes an enterohepatic circulation to be secreted in bile. But, the main route of excretion is via the kidney: urinary excretion of daidzein has been found to account for approximately 30-61% of administered doses [Setchell *et al.,* 2003], the remaining ingested compounds being converted into the metabolites, such as 6,7,4'-trihydroxyisoflavone, 7,3,4'-trihydroxyisoflavone, 7,8,4'-trihydroxyisoflavone, 7,8,3',4'-tetrahydroxyisoflavone, 6,7,8,4'-tetrahydroxyisoflavone, 6,7,3',4'-tetrahydroxyisoflavone, through oxidative pathway [Kulling et al., 2001]. These oxidative seems to have lower bioactivity compared to the microbially reduced products of daidzein [Chang, 2007].

In urine, daidzein exists mainly (~90%) as glucuronides, 7-*O*-monoglucuronides being the most abundant metabolites; free daidzein accounts for 1-10% and the sulfated daidzein about 10% [Shelnutt *et al.*, 2000; Shelnutt *et al.*, 2002]. the level of glucuronides of daidzein is significantly lower in the plasma than in the urine. The sulfate level of daidzeins in the plasma is about two times higher (26%) than that in the urine, while the occurrence of free daidzein is much higher in the plasma than in the urine [Shelnutt *et al.,* 2002; Zhang *et al.,* 2003].

The fecal excretion of daidzein has only recently been investigated. From the data of the few available studies, it is suggested that only about 1% of the ingested daidzein is excreted through the feces. Isoflavones occur mainly in the free aglycone form, with the conjugated isoflavones accounting for less than 10% of the total isoflavones [Adlercreutz *et al.,* 1995; Watanabe *et al.,* 1998; Xu *et al.,* 1995).

Since the discovery of equol in human urine for the first time in 1982 by Axelson *et al.*, daidzein metabolites including DHD, THD, DE, and *O*-desmethylangolensin have been successively detected in the human urine (Table 1). On the basis of these detected metabolites, Joannnou *et al.* [1995] suggested the metabolic pathway of daidzein by human intestinal microflora?. Based on the presence of various reduced metabolites of daidzein, the metabolic sequence prosecuted by the human intestinal bacteria has been proposed; daidzein is reduced to DHD, to THD and dehydroequol, and finally to (3S)-equol in a sequential manner (Fig. 6). However, the pathway and the individual reaction in the pathway are yet to be fully elucidated.

Table 1. Identification of daidzein and its metabolites in human urine

Compound	Year	Reference
Daidzein	1984	Bannwart *et al.*
DHD (Dihydrodaidzein)	1987	Bannwart *et al.*
	1987	Adlercreutz *et al.*
	1993	Kelly *et al.*
	1995	Joannou *et al.*
THD (Tetrahydrodaidzein)	1993	Kelly *et al.*
	1995	Joannou *et al.*
2,3-Dehydroequol	1987	Adlercreutz *et al.*
Equol	1982	Axelson *et al.*
		Adlercreutz *et al.*

Bacterial strains capable of metabolizing daidzein and its derivatives

Although equol has been known to have the highest estrogenic activity among the metabolites of daidzein, only 30 to 40% of humans can produce the equol from daidzein [Kelly *et al.,* 1993; Rafii *et al.,* 2003]. In addition, a high correlation was found between the beneficial effects of the soy food intake and the presence of equol in the urines of the females [Atkinson *et al.,* 2008]. Therefore, the ability to metabolize daidzein into equol conferred by the intestinal microflora in human is regarded as a hallmark of daidzein responsiveness.

In vitro fermentation of daidzein with the human faces samples under anaerobic conditions results in the production of dihydrodaidzein or equol. Setchell *et al.* [1984] demonstrated that incubation of the textured vegetable protein made from soy protein with the cultured human fecal bacteria resulted in the formation of equol. Chang and Nair [1995] also reported the metabolism of daidzein into dihydrodaidzein and equol when incubated with the human faces. Atkinson *et al.* [2004] investigated the metabolism of daidzein by the feces sampled from the equol producer and assessed the effect of antibiotics on the metabolism. The results showed that some antibiotics inhibited the production of equol, but had no effect on the DHD production, suggesting the involvement of many different bacteria in the daidzein metabolism.

Although there have been attempts to isolate the bacterial strains capable of metabolizing daidzein into equol, very little is known about the bacteria responsible for daidzein metabolism in humans. So far, only a few bacterial species have been isolated, characterized, and studied for their activity to metabolize daidzein.

Recent study reported the hydrolysis of the 7-*O*-glycosides daidzein by *Eubacterium ramulus* [Hur *et al.,* 2000a]. The human intestinal bacterium, *Clostrium sp.*

Table 2. Isolated human origin bacterial stains capable of daidzein metabolism

Strains	Activity	Year	Reference
Clostrium Sp. HGH6	Daidzein to DHD	2000	Hur *et al.*
Eubacterium ramulus	Daidzein to *O*-desmethylagolesin	2002	Schoefer *et al.*
Eubacterium ramulus Julong 601	Daidzein to *O*-desmethylagolesin	2004	Wang *et al.*
Eggerthella sp. Julong732	DHD to equol	2005	Wang *et al.*
Clostridium-like strain TM-40	Daidzein to DHD	2007	Tamura *et al.*
Slackia sp. DZE	Daidzein to equol	2008	Jin *et al.*
Eggerthella sp.YY7918	Daidzein to DHD and equol	2008	Yokoyama *et al.*

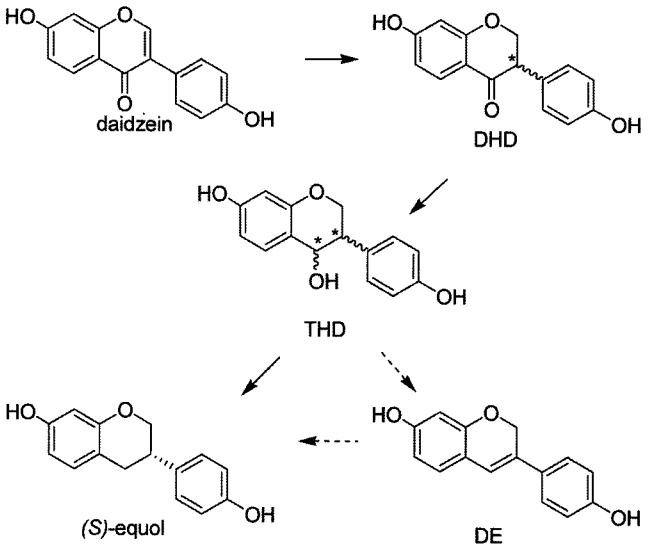

Fig. 6. Proposed pathway for daidzein reduction by intestinal microflora leading to equol formation.

HGH136 [Hur *et al.*, 2002] and *Eubacterium ramulus* Julong 601 [Wang *et al.*, 2004] catalyze the C-ring cleavage of daidzein into nonestrogenic *O*-desmethylangolensin. Two additional strains of the bacteria capable of metabolizing glycoside of daidzein have been isolated from the human faces: *E.Coli* HGH21 (Hur *et al.*, 2000b) and *Clostrium sp.* HGH6 (Hur *et al.*, 2002). However, in spite of their isoflavone daidzein or genistein-specific activity, these strains could not metabolize flavones.

Clostridium-like bacterium strain TM-40 (Tamura *et al.*, 2007) and an anaerobic bacterium *Clostrium sp.* HGH6 (Hur *et al.*, 2000b) reduce the double bond of the isoflavone ring C to yield dihydrodaidzein from daidzein. Recently, an *Eggerthella* strain Julong 732 was found to further reduce DHD into equol [Wang *et al.*, 2005], thus establishing the aforementioned reduction sequence leading to equol from daidzein via DHD in the human intestine [Joannou *et al.*, 1995; Heinonen *et al.*, 1999]. In addition, the human microbial phenotypes that can reduce daidzein all the way to equol were reported [Jin *et al.*, 2008; Yokoyama and Suzuki, 2008]. Nevertheless, the enzymology of the reduction, such as nature of the responsible enzyme and the reaction mechanism, has yet to be established.

The studies on these intestinal microorganisms and the enzymology have been hampered by the strict anaerobic nature of the microorganisms, which calls for the use of special techniques, equipments, and gases. However, the recent availability of the commercial anaerobic chamber would facilitate the investigation on the anaerobes.

Conclusion

The metabolites of daidzein, (3*S*)-equol in particular, play an important role in the human health due to their estrogenic, antioxidative, and anti-cancer activities. It is now recognized that the estrogenic effect, notably aleviation of the post-menopausal syndromes and the prevention of prostate cancer, is due to the (3*S*)-equol that possesses about 100 times higher activity than daidzein. However, the microbiology and biochemistry of the daidzein-metabolizing intestinal bacteria is still in infancy. In light of the importance of equol in the phytoestrogenic effect of the dietary phytoestrogen, the chemistry and enzymology of the DHD reduction to yield (3*S*)-equol should receive extensive attention. For example, the responsiveness toward the dietary daizein intake is directly correlated with the presence of the equol-producing microbe in the intestinal microflora. The evaluation of the intestinal microbes that could convert daidzein into equol would be important in the development of probiotics.

References

Adlercreutz H (2003) Phytoestrogens and breast cancer. *J Steroid Biochem Mol Biol* **83**, 113-118.

Adlercreutz H, Fotsis T, Heikkinen R, Dwyer JT, Woods M, Golin BR, and Gorbach SL (1982) Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and women with breast cancer. *Lancet* **2**, 1295-1299.

Adlercreutz H, Fotsis T, Kurzer MS, Wähälä K, Makela T, and Hase T (1995) Isotope dilution gas chromatographic-

mass spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. *Anal Biochem* **225**, 101-108.

Adlercreutz H, Hockerstededt K, Bannwart C, Bloigu S, Hamalainen E, Fotsis T, and Ollus A (1987) Effect of dietary components, including lignans and phytoestrogens, on enteroheptic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J Steroid Biochem* **27**, 1135-1144.

Akashi T, Aoki T, and Ayage S (1999) Cloning and functional expression of a cytochrome P450 cDNA encoding 2-hydroxyisoflavanone synthase involved in biosynthesis of the isoflavonoid skeleton in licorice. *Plant Physiol* **121**, 821-828.

Akashi T, Aoki T, and Ayage S (2005) Molecular and biochemical characterization of 2-hydroxyisoflavanone dehydratase. Involvement of carbosyesterase-like protein in leguminous isoflavone biosynthesis. *Plant Physiol* **137**, 882-891.

Atkinson C, Berman S, Humbert O, and Lamper JW (2004) In vitro incubation of human faces with daidzein and antibiotics suggests inter individual differences in the bacteria responsible for equol production. *J Nutr* **134**, 596-599.

Atkinson C, Newton KM, Bowles EJA, Yong M, and Lampe JW (2008) Demographic, anthropometric, and lifestyle factors and dietary intakes in relation to daidzein-metabolizing phenotypes among premenopausal women in the United States. *Am J Clin Nutr* **87**, 679-687.

Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson AM, and Setchell KDR (1982) The identification of the weak estrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine. *J Biochem* **201**, 353-357.

Bannwartz C, Adlercreutz H, Fotsis T, Wähälä K, Hase T, and Brunow G (1984) Identification of *O*-desmethylangolensin, a metabolite of daidzein, and of matairesol, one likely plant precursor of the animal lignin enterolactone, in human urine. *Finn Chem Lett* **4-5**, 12-125.

Bannwartz C, Adlercreutz H, Fotsis T, Wähälä K, Brunow G, and Hase T (1987) Identification of the isoflavonic phytoestrogens formononetin and dihydrodaidzein in human urine. *International Symposium on Applied Mass Spectrometry in the Health Sciences.* Barcelona, Spain.

Blount JW, Dixon RA and Paiva NL (1992) Stress responses in alfalfa (*Medicago sativa* L). XVI. Antifungal activity of medicarpin and its biosynthetic precursors; implications for the genetic manipulation of stress metabolites. *Physiol Mol Plant Pathol* **41**, 333-349.

Chang TS (2007) Two potent suicide substrates of mushroom tyrosinase: 7,8,4'-trihydroxyisoflavone and 5,7,8,4'-tetrahydroxyisoflavone. *J Agric Food Chem* **55**, 2010-2015.

Chang YC and Nair MG (1995) Metabolism if daidzein and genistein by intestinal bacteria. *J Nat Prod* **58**, 1892-1896.

Chen Z, Zheng W, Custer LJ, Dai Q, Shu XO, Jin F, and Franke AA (1999) Usual dietary consumption of soy foods and its correlation with the excretion rate of isoflavonoids in overnight urine samples among Chinese women in Shanghai. *Nutr Cancer* **33**, 82-87.

Chin-Dusting JPF, Boak L, Husband A, and Nestel PJ (2004) The isoflavone metabolite dehydroequol produces vasodilatation in human resistance arteries via a nitric oxide-dependent mechanism. *Atherosclerosis* **176**, 45-48.

Choi EJ and Kim GH (2008) Daidzein causes cell cycle arrest at the G1 and g2/M phases in human breast cancer MCF-7 and MDA-BM-453 cells. *Phytomedicine* **15**, 683-690.

Cornwell T, Cohick W, and Raskin I (2004) Dietary phytoestrogens and health. *Phytochemistry* **65**, 995-1016.

Cross HS, Kallay E, Gerdenitsh W, Adlercreutz H, and Armbrecht HJ (2004) Phytoestrogens and vitamin D metabolism: a new concept for the prevention and therapy of colorectal, prostate, and mammary carcinomas. *J Nutr* **134**, 1207S-1212S.

Kulling S, Honig DM, and Metzler M (2001) Oxidative metabolism of the soy isoflavones daidzein and genistein in humans in vitro and in vivo. *J Agric Food Chem* **49**, 3024-3033.

Dixon, RA (2004) Phytoestrogens. *Annu Rev Plant Biol* **55**, 225-261.

Dixon, RA and Sumner LW (2003) Legume natural products: understanding and manipulating complex pathways for human and animal health. *Plant Physiol* **131**, 878-885.

Heinonen S, Wähälä K, and Adlercreutz H (1999) Identification of isoflavone metabolites dihydrodaidzein, dihydrogenistein, 6'-OH-*O*-dma, and *cis*-4-OH-equol in human urine by gas chromatography-mass spectroscopy using authentic reference compounds. *Anal Biochem* **274**, 211-219.

Herman C, Adlercreutz T, Goldin BR, Gorbach SL, Höckerstedt KAV, Watanabe S, Hämäläinen EK, Markkamen MH, Mäkela TH, Wähälä KT, Hase TA, and Rotsis T (1995) Soybean phytoestrogens intake and cancer risk. *J Nutr* **125**, 757S-770S.

Holzbeierlein JM, McIntosh J, and Thrasher JB (2005) The role of soy phytoestrogens in prostate cancer. *Curr Opin Urol* **15**, 17-22.

Hur HG and Rafii F (2000b) Biotransformation of the isoflavonoids biocchanin A, formononetin, and glycitein by *Eubacterium limosum*. *FEMS Microbiol Lett* **192**, 21-25

Hur HG, Berger RD, Heinze TM, Lay JO, Freeman JP, Dore J, and Rafii F (2002) Isolation of anaerobic intestinal bacterium capable of cleaving the C-ring of the isoflavonoid daidzein. *Arch Microbiol* **178**, 8-12.

Hur, HG, Lay JO Jr, Berger RD, Freeman JP, and Rafii F (2000a) Isolation of human intestinal bacteria metabolizing the natural isoflavone glycosides daidzin and genis-

tin. *Arch Microbiol* **174**, 422-428.

Hwang CS, Kwak HS, Lim HJ, Lee SH, Kang YS, Choe TB, Hur HG, and Han KO (2006) Isoflavone metabolites and their in vitro dual functions: They can act as an estrogenic agonist or antagonist depending on the estrogen concentration. *J Ster Biochem Mol Biol* **101**, 246-253.

Jiang F, Jones GT, Husband AJ, and Dusting GJ (2003) Cardiovascular protective effects of synthetic isoflavone derivatives in apolipoprotein E-deficient mice. *J Vasc Res* **40**, 276-284.

Jin JS, Nishihata T, Kakiuchi N, and Hattori M (2008) Biotransformation of *C*-glucosylisoflavone puerarin to estrogenic (3*S*)-equol in co-culture of two human intestinal bacteria. *Biol Pharm Bull* **31**, 1621-1625.

Joannou GE, Kelly GE, Reeder AY, Waring M, and Nelson C (1995) A urinary profile study of dietary phytoestrogens, the identification and mode of metabolism of new isoflavonoids. *J Ster Biochem Mol Biol* **54**, 167-184.

Kelly GE Joannou GE, Reeder AY, Nelson C, and Waring MA (1995) The variable metabolic response to dietary isoflavones in humans. *Proc Soc Exp Biol Med* **208**, 40-43.

Kelly GE, Nelson C, Waring MA, Joannou GE, and Reeder AY (1993) Metabolites of dietary (soya) isoflavones in human urine. *Clin Chim Acta* **223**, 9-22.

Kim J and Kwon C (2001) Estimated dietary isoflavone intake of Korean population based on national nutrition survey. *Nutr Res* **21**, 947-953.

Kanellakis P, Nestel P, and Bobik A (2004) Angioplasty-induced superoxide anion and neointimal hyperplasia in the rabbit carotid artery; suppression by the isoflavone trans-tetrahydroddaidzein. *Antherosclerosis* **176**, 63-72.

Kelly GE, Nelson C, Waring MA, Joannou GE, and Reeder AY (1993) Metabolites of dietary (soya) isoflavones in human urine. *Clin Chim Acta* **223**, 9-22.

Kirk EA, Sutherland P, Wang SA, Chait A, and LeBoeuf RC (1998) Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *J Nutr* **128**, 954-959.

Lee HP, Gourley L, Duffy SW, Estève J, Lee J, and Day NE (1991) Dietary effects on breast-cancer risk in singapore. *Lancet* **337**, 1197-1200.

Liggins J Bluck LJC, Runswick S, Atkinson C, Coward WA, and Bingham SA (2000) Daidzein and genistein content of fruits and nuts. *J Nutr Biochem* **11**, 326-331.

Ling S, Dai A, Williams MRI, Husband AJ, Nestel PJ, Komesaroff PA, and Sudhir K (2004) The isoflavone metabolite *cis*-tetrahydrodaidzein inhibits ERK-1 activation and proliferation in human vascular smooth muscle cells. *J Cardiovasc Pharm* **43**, 622-628.

Liu CJ, Blount JW, Steel CLe, and Dixon RA (2002) Bottlenecks for metabolic engineering of isoflavone glycoconjugates in Arabidopsis. *Proc Natl Acad Sci USA* **99**, 14578-14583.

Marais JPJ, Deavour B, Dixon RA, and Ferreira D (2006) The stereochemistry of flavonoids In *The Science of Flavonoids* Groteword E (ed) pp.1-46 Springer, NY.

Matsumura A Ghosh A, Pope GS, and Dabre PD (2005) Comparative study of estrogenic properties of eight phytoestrogens in MCF7 human breast cancer cells. *J Ster Biochem Mol Biol* **94**, 431-443

Mazur W and Adlercruetz H (1998) Naturally occurring estrogens in food. *Pure Appl Chem* **70**, 1759-1776.

Mazur W and Adlercreutz, H (2000) Overview of naturally occurring endocrine-active substances in the human diet in relation to human health. *Nutrition* **16**, 654-658.

Nestel P, Fujii A, and Zhang L (2007) An isoflavone metabolite reduces arterial stiffness and blood pressure in overnight men and postmenopousal women. *Antherosclerosis* **192**, 184-189.

Shelnutt SR, Cimino CO, Wiggins PA, and Badger TM (2000) Urinary Pharmacokinetics of the Glucuronide and Sulfate Conjugates of Genistein and Daidzein. *Cancer Epidemiol Biomarkers Prev* **9**, 413-419.

Shelnutt SR, Cimino CO, Wiggins PA, Ronis MJJ, and Badger TM (2002) Pharmacokinetics of the glucuronide and sulfate conjugates of genistein and daidzein in men and women after consumption of a soy beverage. *Am J Clin Nutr* **76**, 588-594.

Setchell KD, Borriello SP, Hulme P, Kirk DN, and Axelson M (1984) Non-steroidal estrogens of dietary origin: possible roles in hormonedependent disease. *Am J Clin Nutr* **40**, 569-578.

Setchell KDR (1998) Phytoestrogens: the biochemistry, and implications for human health of soy isoflavones. *Am J Clin Nutr* **68**, 1333S-1346S.

Setchell KDR and Cassidy A (1999) dietary isoflavones: biological effects and relevance to human health. *J Nutr* **129**, 758-767.

Setchell KDR, Faughnan MS, Avades T, Zimmer-Nechemias L, Brown NM, Wolfe BE, Brashear WT, Desai P, Oldfield MF, Botting NP, and Cassidy A (2003) Comparing the pharmacokinetics of daidzein and genistein with the use of ^{13}C-labeled tracers in premenopausal women. *Am J Clin Nutr* **77**, 411-419.

Tamura M, Tsushida T, and Shunohara K (2007) Isolation of isoflavanone-metabolizing, *Clostridium*-like bacteria, strain TM-40, from human feces. *Anaerobe* **13**, 32-35.

Tian L and Dixon RA (2006) Engineering isoflavone metabolism with an artificial bifunctional enzyme. *Planta* **224**, 496-507.

Verma SP and Goldin BR (1998) Effect of soy-derived isoflavonoids on the induced growth of MCF-7 cells by estrogenic environmental chemicals. *Nutr Cancer* **30**, 232-239.

Wähälä K, Koskimies JK, Mesilaakso M, Salakka AK, Leino TK, and Adlercreutz H (1997) The synthesis, structure, and anticancer activities of cis-and trans-4,',7-dihydroisoflavan-4-ols. *J Org Chem* **62**,7690-7693.

Wakai K, Egami I, Kato K, Kawamura T, Tamakosh A, Lin Y, Nakayama T, Wada M, and Ohno Y (1999) Dietary

intake and sources of isoflavanones among Japanese. *Nutr Cancer* **33**, 139-145.

Wang HJ and Murphy PA (1994) Isoflavone content in commercial soybean foods. *J Agric Food Chem* **42**, 1666-1673.

Wang XL, Kim KT, Lee JH, Hur HG, and Kim SI (2004) C-ring cleavage of isoflavones daidzein and genistein by a newly-isolated human intestinal bacterium Eubacterium ramulus Julong 601. *J Microbiol Biotechnol* **14**, 766-771.

Wang XL, Hur HG, Lee JH, Kim KT, and Kim SI (2005) Enantioselective synthesis of *S*-equol from dihydrodaidzein by a newly isolated anaerobic human intestinal bacterium. *Appl Environ Microbiol* **71**, 214-219.

Watanabe S, Yamaguchi M, Sobue T, Takahashi T, Miura T, Arai Y, Mazur W, Wähälä K, and Adlercreutz H (1998) Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (Kinako). *J Nutr* **128**, 1710-1715.

Winkel-Shirley B (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol* **126**, 485-493.

Won D, Shin BK, Kang S, Hur HG, Kim M, and Han J (2008) Absolute configurations of isoflavan-4-ol sterioisomers. *Bioorg Med Chem Lett* **18**, 1953-1957.

Xu X, Harris KS, Wang HJ, Murphy PA, and Hendrich S (1995) Bioavailability of soybean isoflavones depends upon gut microflora in women. *J Nutr* **125**, 2307-2315.

Yokoyama S and Suzuki T (2008) Isolation and characterization of a novel equol-producing bacterium from human feces. *Bioscie Biotechnol Biochem* **72**, 2660-2666.

Yu O, Jung W, Shi J, Croes RA, Fader GM, Mcgonigle B, and Odell JT (2000) Production of the isoflavone genistein and daidzein in non-legume dicot and monocot tissue. *Plant Physiol* **124**, 781-793.

Yu O, Shi J, Hesion AO, Maxwell CA, Mcgonigle B, and Odell JT (2003) Metabolic engineering to increase isoflavone biosynthesis on soybean seed. *Phytochemistry* **63**, 753-763.

Zhang Y, Hendrich S, and Murphy PA (2003) Glucuronides are the main isoflavone metabolites in women. *J Nutr* **133**, 399-404.