CHAPTER 1
INTRODUCTION

Spirogyra neglecta (SN) or common known in Thai as "*Tao*", is a genus of filamentous green algae of the order Zygnematales. It has unique characteristics by a helical or spiral arrangement of the chloroplasts. It is approximately 10 to 100 μm in width and the length stretches up to centimeters long. SN has been widely grown in the Nan River, Northern Thailand. In *vivo* studies indicated that this species has several beneficial effects, including anti-gastric ulcer, anti-inflammatory, anti-hyperglycemic and anti-hyperlipidemic actions (Amornlerdpison et al., 2011; Lailerd et al., 2010; Peerapornpisal et al., 2008). Recently, antioxidant effect of SN has been shown *in vitro* and it was suggested that SN may subsequently be useful for applying as therapeutic drugs and/or food supplements (Peerapornpisal, 2007). However, the information concerning its effects and the mechanisms of its action on particular diseases remains unknown.

Diabetes mellitus (DM) is possibly the world's largest growing metabolic disease (Baily and Flatt, 1944). Broadly, it is classified into three categories: Type 1 diabetes (T1DM) accounts for 10% of diabetes. The etiology is by autoimmune destruction of pancreatic β-cells, leading to insulin deficiency (Lailerd et al., 2010) whereas type 2 diabetes (T2DM) is mostly resulted from the combination of insulin resistance and/or β-cells secretory defect. Less than 10% of diabetes is classified as the third class, which is caused by genetic defect of β-cell, impairment of insulin production and its function, exocrine and endocrine defects, drug induced-aberration, and gestational diabetes (Association, 2001; Cnop et al., 2005; Singh, 2009). It is known that T2DM is normally characterized by the excessive hepatic glucose production, decreased insulin secretion, and insulin resistance (DeFronzo, 1997; Grodsky, 2000; Kahn, 1994; Porte, 2001; Reaven, 2000). Previous studies have shown that T2DM is related to the development of diabetic complications, including nephropathy, neuropathy and retinopathy (Association, 2001, 2004a; Diederich et al., 1994; Sanz et al., 2003; Zimmet et al., 1997). For 30-40% of T2DM patients who

develop diabetic nephropathy, the kidney gradually lose the ability to function properly, leading to end-stage renal disease (ESRD) (Jindal et al., 2005). Therefore, diabetic nephropathy has become the most frequent condition, which could lead to the excretion of high amount of protein in the urine and finally develop renal failure (Association, 2004b; Manna et al., 2009). Furthermore, these complications have been found to involve with the generation of reactive oxygen species (ROS) and lead to oxidative stress condition as it was shown that the level of antioxidants was decreased in 8 weeks of diabetic nephropathy rats (Ali Taghizadeh Afshari 2007; Gross et al., 2005). Several studies have been shown the mechanisms of DM or hyperglycemia induced ROS production. For instance, the activation of protein kinase C (PKC) and/or mitogen-activated protein kinase (MAPK), and advanced glycation end-products (AGE) generation by increasing hydrogen peroxide in mesangial cells leading to increased lipid peroxidation in glomeruli (Baynes, 1991; Makino et al., 1995; Park et al., 2001; Studer et al., 1997).

The kidneys are known to play an important role in the elimination of several xenobiotics, including drugs, toxins, and endogenous compounds (Vincent et al., 2006). The active secretion of anionic substances to the tubular lumen appears to be restricted to the basolateral membrane of proximal tubule via several transporters. At the present, membrane transporters involved in the renal tubular secretion have been cloned and identified (Srimaroeng et al., 2008; Tanaka et al., 2004). Organic anion transporters 1 and 3 (Oat1 and Oat3) have been shown to play a major role in the cellular uptake of organic anions across the basolateral membrane of renal proximal tubules resulted in organic anion excretion (Soodvilai et al., 2004). Renal Oat1 and Oat3 belong to organic anion transporter family encoded by SLC22A. Their proteins consist of 536-562 amino acids (Hosoyamada et al., 1999; Lu et al., 1999; Race et al., 1999; Reid et al., 1998). Secondary structure based on hydropathy analysis indicates that Oat1 and 3 contain 12 transmembrane domains (TMDs) with 4 potential N-glycosylation sites and 3-8 potential phosphorylation sites for PKC, depending upon species (Burckhardt and Wolff, 2000; Cha et al., 2001; Kusuhara et al., 1999). Rat Oat1 and 3 mRNA are highly expressed in the kidneys, liver, brain and eyes (Buist and Klaassen, 2004). Like Oat1, Oat3 recognizes a broad spectrum of substrates, and it mediates the high-affinity transport of several substrates, including estrone sulfate

(ES), specific substrate of Oat3, *para*-aminohippurate (PAH), ochratoxin A, and various anionic drugs in exchange for dicarboxylates inside the cells (Sweet et al., 2003). The classical mechanism of basolateral organic anion exchanger is a tertiary active transport, depends upon an inward-directed Na^+ gradient to drive the uptake of α-ketoglutarate (α-KG), which is then exchanged for organic anions (Burckhardt and Burckhardt, 2003; Dantzler, 1996; Friis, 1991; Pritchard, 1992). Regarding to the regulation of transporters, several compounds have also shown to modulate basolateral Oat1 and Oat3 expressions and functions. Bradykinin has shown to decrease in both estrone sulfate (ES) transport and hOAT3 expression (Li et al., 2010). Moreover, hOAT1 expressed in both stable cell lines and *Xenopus* oocytes were down-regulated by either angiotensin II or PKC activator, phorbol ester (PMA) (Wolff et al., 2003).

Recent data indicated that the expression and function of organic anion transporters can be regulated by pathophysiological status. For example, an increase in the amount of indoxyl sulfate in proximal tubular cells led to an increase the expression of Oat1 and Oat3 in chronic renal failure in rats (Enomoto et al., 2002). Bilateral urethral obstruction and renal ischemic/reperfusion injury in adult male rats decreased mRNA and protein expression of both Oat1 and Oat3 in basolateral membrane fractions (Schneider et al., 2007; Villar et al., 2005). In addition, human OAT1 and OAT3 were down-regulated in chronic renal failure, leading to impaired renal secretion of cytotoxic metabolites; cefazolin and phenolsulfonphthalein, and caused glutarate derivatives; αKG , L-2OHGA, D-2OHGA, 3OHGA, glutaconate, and adipate accumulations as well as mitochondria dysfunction (Sakurai et al., 2004; Sauvant et al., 2006). Furthermore, previous study found that the expression of rat organic cation transporter (rOCT) was reduced in proportion to tubular injury in T1DM whereas mOat3 expression and function were decreased in streptozotocin induced T1DM (Lungkaphin et al., 2012; Thomas et al., 2003). However, there is no information regarding to the expression and function of Oat1 and 3 in T2DM. The implication between ROS and several transporters was recently shown. The patients with chronic hepatitis C viral infection exhibited elevated levels of circulating ROS, leading to the decrease of Oct1 mRNA levels (Nakai et al., 2008). Furthermore, ROS induced by renal ischemic/reperfusion injury directly affected the regulation of Oat1,

Oat3, Oct2 and Multidrug and toxin extrusion 1 (MATE1) proteins in rats (Ikemura et al., 2009; Matsuzaki et al., 2008; Matsuzaki et al., 2007).

Taken together, it is likely that the presence of ROS in diabetes associates with the expression and function of transporters and because of *in vitro* study have shown antioxidant activity of *Spirogyra neglecta* extract. Therefore, we aimed to examine the effect and identify the mechanism of SN extract on the functions of rat Oat1 and Oat3 (rOat1and rOat3) in T2DM rats. The data obtained from this study could provide the beneficial information of SN supplement on rOat1 and 3 transport functions in T2DM rats. In addition, the new findings from this study could be transferred into clinical application by improving the therapeutic approach for T2DM and other ROS-associated diseases using Thai natural supplement.

LITERATURE REVIEW

Spirogyra neglecta (SN)

The Biological source of SN

SN or common name in Thai "Tao" is a genus of filamentous green macroalgae, named for its helical or spiral arrangement of the chloroplasts. The vegetative diameter is approximately 52-65 µm. This algae has 10 to 100 µm in width and may stretch up to centimeters long (Figure 1) (John Whitton and Brook, 2002; Naskar, 2009).

The taxonomic position of SN is as follows (Bold and Wynne, 1978; Punyoyai, 2008):

Kingdom Protista
Division Chlorophyta
Class Zygnemaphyceae
Order Zygnematales
Family Zygnemataceae
Genus Spirogyra

Scale bar = 50µm

Figure 1 Fresh *Spirogyra neglecta* (a) Picture from field, filamentous green algae commonly found in fresh water as skeins of fine green threads. (b) Image under the microscope (40X), inside each cell is one or more ribbon-like, spirally arranged chloroplast(s), called Spirogyra (Peerapornpisal, 2007; Punyoyai, 2008)

This filamentous algae is un-branched with cylindrical cells and covered by mucilage (Bold and Wynne, 1978). The cell wall has 3 layers where the inner and middle layers are mainly composed of cellulose whereas the outer contains pectose. The cytoplasm forms a thin lining underneath cell walls and the nucleus suspends in the cell by cytoplasm strands. Chloroplasts are embedded in the peripheral cytoplasm by one or variable numbers. The chloroplasts are in the ribbon shaped, serrated, scalloped, and spirally arranged, showing green spiral characteristic on each filament. Each chloroplast contains several pyrenoids, centers for the production of starches, appearing as small round bodies (Figure 2) (Giordano et al., 2005; Hanson et al., 2002).

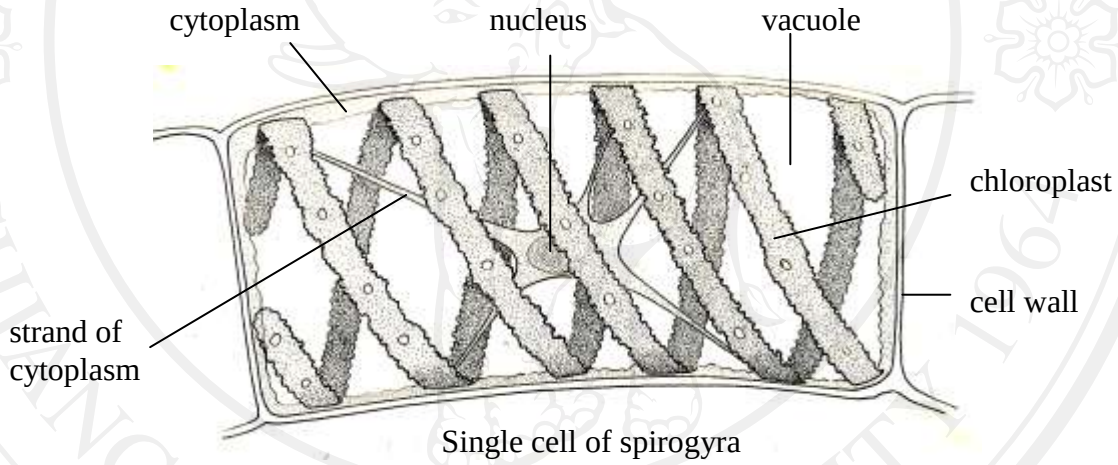

Figure 2 Cell structure of *Spirogyra neglecta* (Mackean, 2002)

SN has natural pigments that composes of chlorophylls (A and B), carotene (alpha (α), beta (β) and gamma (γ)), xanthophylls (lutien and violaxanthin) and neoxanthin in different amounts, depending on the type of algae, living environment, and light exposure (Karnjanaparch, 1984).These pigments have also shown antioxidant properties (Kranner et al., 2002). In addition, it grows under eutrophic water at 15-27 °C, pH in the range of 6 - 7.8 and the turbidity under 10 NTU (Peerapornpisal, 2007). SN contains various kinds of nutrients (Table 1), therefore, it has been suggested to apply as food supplement and/or therapeutic regimen due to its nutritional, pharmaceutical, bioactive and antioxidative advantages.

Table 1 The nutritional compositions of *Spirogyra neglecta* (Peerapornpisal, 2007)

The studied parameters	Amount
Basic nutrients (g% dry weight)	
Fat	5.21
Protein	18.65
Fiber	7.66
Ash	11.78
Carbohydrate	56.31
Vitamins (mg% dry weight)	
Vitamin A	0.25
Vitamin B1	0.04
Vitamin B2	0.55
Niacin	3.65
Minerals (mg% dry weight)	
Calcium	26.88
Sodium	1.56
Potassium (g% dry weight)	0.00119
Magnesium	241.10
Manganese	35.80
Iron	33.85

In a phytochemical screening study by Amornlerdpisarn, D., et al. 2011 (Amornlerdpison et al., 2011), 1 g of SN extract contained polyphenolic compounds which has antioxidative activity equivalent to that extracted from 77.7 $\pm$ 3.6 mg GAE per gram extract, the standard control of antioxidation (Peerapornpisal et al., 2009). The ratio of the phenolic compounds in SN extract and gallic acid was previously used in gallic acid equivalent (GAE) unit (Punyoyai, 2008). Besides polyphenolic compounds, SN extract also has sulfate and polysaccharide contents for 1.1 ± 0.01% and 33.9 ± 1.5%, respectively (Amornlerdpison et al., 2011).

Biological activities of *Spirogyra neglecta*

1. Anti-inflammatory action

SN was recently found to have an anti-inflammatory effect (Amornlerdpison et al., 2011). It has shown that rat ear edema and inflammation induced by ethyl phenylpropiolate (EPP) were reduced after acute application of SN extract at the dose 2.5 mg/ear for 1-2 hours and at the dose of 5.0 mg/ear for 0.5-1 hours. Moreover, the degree of gastric ulcer in rats induced by either the restraint water immersion stress, mixture of HCl/ethanol or indomethacin were significantly reduced after oral administration of SN at the doses of 500 mg/kg body weight (BW)/day for 1 hr, indicating gastro-protective effect of SN in gastric ulcer-induced rat model(Amornlerdpison et al., 2011).

2. Antioxidant action

Previous study has shown that SN aqueous extract at the dose of 0.01-1.5 mg/ml was markedly decreased radical scavenging activities *in vitro* using lipid peroxidation, superoxide ($O_2^{-\bullet}$) and hydroxyl ($OH^{\bullet}$) radicals scavenging assays (Peerapornpisal et al., 2008). In addition, water extract of SN had shown the highest antioxidant activity than other 2 species of macroalgae, which were *Cladophora spp. and Nostochopsis spp* by 61 and 10.3 folds of standard trolox equivalent antioxidant capacity (TEAC) µmol/g, respectively (Peerapornpisal, 2007).

3. Anti-hyperglycemia and anti-hyperlipidemia actions

More recently, it has shown that 1000 mg/kg BW of SN extract supplementation in T2DM rats for 12 weeks were significant reduced plasma glucose and free fatty acid for 50 and 27%, respectively (Lailerd et al., 2010).

4. Toxicological indicator

The study of sorption properties of heavy metal ions (Mn, Cu, Zn and Cd) suggested that SN was an indicator of polluted and turbulent water, and became a biomonitor as it is widely grown and its great ability to adapt in various environmental systems (Rajfur et al., 2010; Venketshwarlu and Reddy, 1997).

Diabetes Mellitus

Definition

DM is the one of metabolic diseases characterized by hyperglycemia, which leads to either insufficient or absent of insulin production from the pancreas, insulin resistance at target receptors, and imbalance in carbohydrate and lipid metabolisms. The symtoms are often accompanied by glycosuria, polydipsia and polyuria (Mushtaq, 2009). Moreover, chronic hyperglycemia is mostly associated with long-term damage, dysfunction and failure of various tissues, especially the eyes, kidneys, nerves, heart and blood vessels (Mustafa et al., 2002; Sarika, 2010).

Classification of Diabetes mellitus

DM is currently classified into three categories: type 1 diabetes (T1DM) is previously known as insulin-dependent or childhood-onset diabetes. This type is accounted for 10% of diabetes and it is caused by autoimmune destruction of pancreatic beta (β) cells, resulting in an insufficiency of insulin production. Secondly, type 2 diabetes (T2DM) is also named as non-insulin-dependent or adult-onset diabetes that is about 80-90% of diabetes. The major cause of T2DM is mostly from the combination of insulin resistance and β-cells secretory defect. The last type is found less than 10% by different causes, such as, genetic defects of β-cell, impairment of insulin production and its function, exocrine and endocrine defects, drug induced aberrations or gestational diabetes (Association, 2001; Cnop et al., 2005; Singh, 2009).

T1DM is commonly found in children as an autoimmune disease, which usually leads to absolute insulin deficiency due to the loss of insulin-producing from β-cells of the islets of Langerhans (Venketshwarlu and Reddy, 1997). The onset is usually acute, developing over a period of a few days to weeks. Most of these patients are involved with the β-cell loss and/or T-cell begins to release cytokines that stimulate inflammation (Ferrara, 2000). Sensitivity and responsiveness of T1DM patients to insulin are usually normal, especially in the early stages (Mellitus, 1980).

T2DM is characterized by excessive hepatic glucose production, decrease insulin secretion, and insulin resistance (DeFronzo, 1997; Kahn, 1994; Porte, 2001;

Reaven, 2000). The presence of insulin resistance indicated by both reduction of glucose disposition in skeletal muscles and suppression of endogenous glucose production, primarily in the liver(Dinneen et al., 1992). Recently, it has been indicated that approximate 25% of non-diabetic patients also exhibited insulin resistance within the same range of insulin level that can be observed in patients with T2DM (Hollenbeck and Reaven, 1987; Reaven et al., 1993; Reaven et al., 1989). Furthermore, insulin resistance was also found to be associated with several metabolic abnormalities such as central obesity, hypertension, and dyslipidemia, suggesting that these factors contribute to a high rate of cardiovascular morbidity and mortality in human and animal models (Evans et al., 2002; Meis et al., 2006).

Diabetes mellitus and oxidative stress

It is uncertain between hyperglycemia and oxidative stress relationship. Generally, normal cells are able to defend themselves against free radical production through three primary scavenger enzymes, which are catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) (Mates et al., 1999; Moussa, 2008). However, previous studies have shown that diabetes that characterized by persistent hyperglycemia, might cause an increase in high amount of free radical production. An imbalance between the high production of free radical-generating substances, especially reactive oxygen species (ROS), and the reduction of radical-scavenging enzymes, was suggested to produce oxidative stress (Moussa, 2008).

Hyperglycemic condition produced oxidative stress due to increased production of mitochondrial ROS (Evans et al., 2002). The excessive glucose reached the mitochondria, leading to an overdrive of the electron transport chain and subsequently produced excessive ROS while various enzymes, including NADPH oxidase, xantine oxidase and nitric oxide synthase (NOS), were showed to up-regulate in both streptozotocin (STZ)-induced diabetic (Hink et al., 2001; Shinozaki et al., 1999) and fructose-fed induced hyperinsulinemic rats (Shinozaki et al., 1999). Furthermore, the auto-oxidation of glucose, non-enzymatic glycation of the proteins, and the polyol pathway were indicated to be potential sources of hyperglycemia-induced oxidative stress. ROS has shown to damage several components of the cells, including phospholipids of endoplasmic reticulum and plasma membrane, poly-

unsaturated fatty acids of mitochondria, and nuclear and mitochondria DNA of liver, brain and heart tissues (Melov, 2000; Melov et al., 1999).

Oxidative stress and stress-sensitive pathway

The cellular mechanisms of diabetes induced oxidative stress have been recently proposed. The activation of stress sensitive pathways, including nuclear factor keppa B (NF-κB), P38 mitogen-activated protein kinases (p38), mitogen-activated protein kinase (MAPK), NH2-terminal Jun kinases/stress-activated protein kinases (JNK/SAPK), advanced glycation end-products (AGE), receptor for AGE (RAGE), and protein kinase C (PKC) were suggested to interfere with several gene expressions, resulting in cellular damages (Allen and Tresini, 2000; Evans et al., 2002; Maritim et al., 2003; Welborn et al., 1998). In addition, activation of these stress-sensitive signaling pathways was also showed to be involved with insulin resistance and impaired insulin secretion conditions (Evans et al., 2002). Several studies have shown that T2DM activated oxidative stress related to development of diabetic complications include neuropathy, retinopathy and nephropathy (Association, 2001; Diederich et al., 1994; Sanz et al., 2003; Zimmet et al., 1997).

As mentioned above, one of the pre-dominant factor induced oxidative stress was the activation of PKC**.** Protein kinase C is a family of serine/threonine-specific protein kinases, consisting of several isoforms in different subclasses (Nishizuka, 1992). Each subclass has been categorized by their potential domains, including regulatory and catalytic domain (Inoguchi et al., 1992). Classical PKCs, which are PKCα, β, γ contained both regulatory and catalytic domains. Most of regulartory domain structure of classical PKC plays important role for calcium binding that subsequently transduced intracellular signaling cascade. For instance, bradykinin and angiotensin II have demonstrated to activate PKCα (Nishizuka, 1995). A novel PKC composed of regulatory and catalytic domains that critical for DAG binding while atypical PKCs as known as PKCζ and λ have only catalytic domains that had also shown to activate organic anion transporter 1 and 3 (Oat1 and 3) (Barros et al., 2009).

More recently, it was revealed that hyperglycemia induced the accumulation of diacylglycerol (DAG), and activated classical PKCs in cultured mesangial cells and explants of rat glomeruli (Meier and King, 2000). Elevated glucose in the renal tissues

caused increased concentration of glycolysis metabolites, leading to de novo synthesis of DAG. These consequences direct or indirect activated several PKC isoforms including, PKC α, δ, and ε resulted in their translocation to nucleus (Kapor-Drezgic et al., 1999) while PKCα and ζ translocated to the membrane and subsequently impaired antioxidant defense system (Figure 3) (Haneda et al., 1995; Kapor-Drezgic et al., 1999). Furthermore, ROS or advanced glycation end products (AGEs) could also prolong activated PKC by stimulated vascular endothelial growth factor (VEGF) expression, indicating a role of ROS acted as a key mediator for AGE-induced PKC activation (Mamputu and Renier, 2002; Scivittaro et al., 2000). Therefore, the activation of the PKC induced by hyperglycemia might be a major factor to contribute diabetic complication.

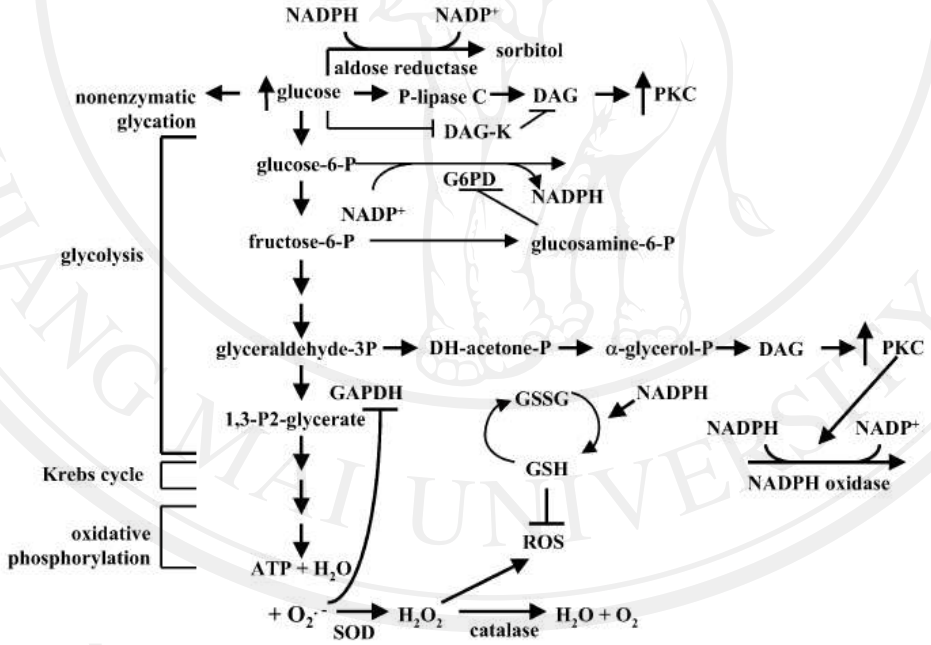

Figure 3 Diagram illustrates of several pathways that contribute to oxidative stress in response to increased glucose flux. Increased glucose flux both enhances oxidant and reactive oxygen species production and impairs antioxidant defenses through PKC activation by multiple interacting pathways, including de novo synthesis of diacylglycerol (DAG), activation of phospholipase C, or inhibition of DAG kinase (King and Loeken, 2004).

Diabetic nephropathy

Diabetic nephropathy is a progressive kidney disease caused by angiopathy of glomerular capillaries that are characterized by persistent albuminuria (>300 mg/24 hr or 200 μg/min), early elevation of arterial blood pressure, a relentless decline in renal function, and increased cardiovascular morbidity and mortality (Rossing, 2006). Previous studies have revealed that duration of at least 25 years in T1DM and T2DM showed a cumulative risk of diabetic nephropathy for 25–40% after diabetes (Andersen et al., 1983; Ballard et al., 1988; Wetzels et al., 1986). For 30-40% of T2DM patients who develop diabetic nephropathy, the kidneys were gradually lost their ability to function properly. It initially developed mesangial expansion due to enhancement of mesangial matrix deposition and subsequently mesangial cells and basement membrane hypertrophy (Alsaad and Herzenberg, 2007; Donnelly, 2005; Flyvbjerg, 2006). In addition, glomerular filtration rate was high due to plasma flow rate and capillary pressure increased as well as the overproduction of prostaglandin E2 (PGE2), suggesting that diabetic nephropathy caused by hemodynamic alteration, which could appear in both T1DM and T2DM (Baykal-Erkilic et al., 1995; Makino et al., 2002; Mogensen and Andersen, 1975). Previous studies found that tumor growth factor β1 (TGFβ1) and tumor necrosis factor α (TNFα) gene expressions were increased (Navarro et al., 2005; Sharma and Ziyadeh, 1994) and several proteins from stress-sensitive protein pathway, including AGE, sorbitol, PKC, growth factor and cytokines (e.g. interleukin-1β) were activated in diabetic nephropathy rats (Parving et al., 2000) whereas it became more progressive when macro-albuminuria or proteinuria was presented (Themis et al., 2009). Moreover, the early stage of diabetic nephropathy rats was characterized by a small increase in urinary albumin excretion (microalbuminuria) due to afferent and efferent hyaline arteriosclerosis resulted in changing renal haemodynamic as well as ultimate interstitial fibrosis and tubular atrophy (Wolf, 2004). Those factors also finally led to end stage renal failure (ESRF) in human (Alsaad and Herzenberg, 2007). Therefore, progressive nephropathy is frequently found as a common consequence of long-term DM that involved in both glomerular alteration and obviously structural changed in proximal tubule (Phillips, 2003).

Organic Anion Transporters

For more than a decade, many renal tubular drug transporters have been extensive studied. Among these transporters, the majority is the solute carrier 22A family (SLC22A family), which consists of organic anion transporters (OATs) and organic cation transporters (OCTs) (Roch-Ramel et al., 1992; Ullrich, 1994). Several of these transporters are localized at the basolateral membrane of proximal tubules and have been suggested to mediate basolateral uptake of various ionic compounds. At the present, organic anion transporter 1 and 3 (Oat1 and Oat3) have been revealed to play a major role in the cellular uptake of organic anions across the basolateral membrane of renal proximal tubules in several species including mouse, rat, and human (Brady et al., 1999; Kusuhara et al., 1999; Race et al., 1999; Soodvilai et al., 2004; Srimaroeng et al., 2008).

Oat1 protein consists of 551-562 amino acids (Hosoyamada et al., 1999; Lu et al., 1999; Race et al., 1999) with secondary structure of 12 transmembrane domains (TMDs), with cytoplasmic amino and carboxyl terminals based on hydropathy analysis. The large extracellular loop between TMD 1 and 2 contains 3-6 potential N-glycosylation sites, depending on species. Potential phosphorylation sites for PKC, protein kinase A (PKA), casein kinase II, and tyrosine kinase are clustered in the intracellular loop between TMD 6 and 7 and on the carboxyl terminus (Figure 4) (Koepsell and Endou, 2004). Furthermore, its substrates include *para*-amino-hippuric acid (PAH), a prototypic substrate that could be completely cleared from the renal plasma by a single pass (Pritchard and Miller, 1993), cyclic nucleotides, prostaglandins, and urate as well as exogenous compounds, such as, diuretic drugs (carbonic anhydrase inhibitors, loop diuretics and thiazide) and endogenous compounds such as cyclic AMP (cAMP), cyclic GMP (cGMP), and PGE2. Several organic anions could decrease Oat1-mediated substrate transport, including antibiotics; penicillines, cephalosporines, quinolones, aminoglycoside, antivirals drugs; acyclic nucleoside analogues, nucleoside analogues, nucleotide analogues, anti-inflammatory drugs, chemotherapeutic drugs, vitamins, and anti-hypertensive drugs (Burckhardt and Burckhardt, 2011; Sekine et al., 2000; Srimaroeng et al., 2008).

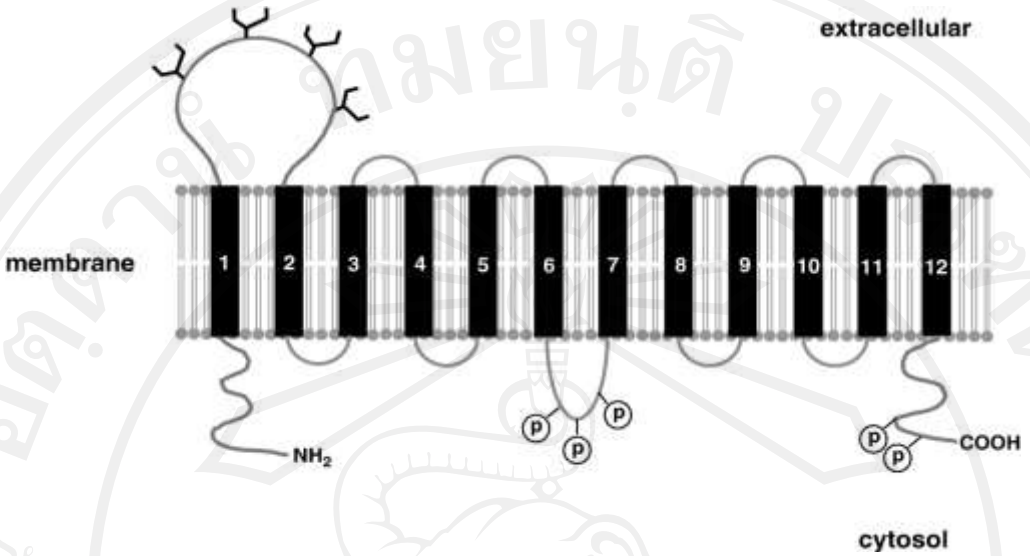

Figure 4 Predicted transmembrane topology of OAT family. Twelve transmembrane domains are numbered from 1 to 12. Potential glycosylation sites are denoted by tree-like structures. Potential phosphorylation sites are labeled as "P" (Duan and You, 2010).

Similar to Oat1, Oat3 protein consists of 536-542 amino acids with 12 TMDs and 4 potential N-glycosylation sites, depending upon species. It has 3-8 potential phosphorylation sites for PKC (Burckhardt and Wolff, 2000; Cha et al., 2001; Kusuhara et al., 1999). Oat1 and Oat3 mRNA are mainly expressed in the kidneys, liver, brain and eyes (Kusuhara et al., 1999). Oat3 recognizes a broad spectrum of substrates and the substrate specificities of Oat1 and 3 are overlapped but not identical. It mediates the high-affinity transport of estrone sulfate (ES), which is its specific substrate, PAH, ochratoxin A, taurocholate, glutarate, the endogenous compounds; cAMP and cGMP, cortisol and PGE2. A wide range of drugs could also inhibit Oat3-mediated ES uptake, including benzylpenicillin. It has been shown that a weak substrate transport by human OAT3 were cidofovir, tenofovir, valacyclovir, H2 antagonists, non-steroidal anti-inflammatory drugs (NSAIDs), diuretics, anti-epileptics, anti-neoplastics, and probenecid (Burckhardt and Burckhardt, 2003; Burckhardt and Burckhardt, 2011; Sweet et al., 2003).

Mechanism of trans-epithelial organic anion transport

The process of trans-epithelial renal tubular secretion of anionic compounds from blood circulation to the renal tubular lumen has been well-characterized and shown to be involved with two steps (Figure 5) (Soodvilai et al., 2004). Initially, anionic substrates are transported from blood to the renal epithelial cells across the basolateral membrane against an electrochemical gradient. This step is the most important process as it is a rate-limiting step of renal trans-epithelial transport and this has been called as "tertiary active transport process"(Sekine et al., 1997; Sweet et al., 1997). Subsequently, anionic compounds move from inside the cells to the lumen down an electrochemical gradient, presumably by facilitated diffusion (Pritchard and Miller, 1993; Wright and Dantzler, 2004). As shown in Figure 5, tertiary active transport begins with the counter transport of an organic anion into the cell against its electrochemical gradient in exchange for the efflux of alpha-ketoglutarate (α-KG) by Oats. The outwardly directed gradient for α-KG is not only maintained by intracellular metabolic generation of α-KG but also fueled by active α-KG uptake across the basolateral membrane via a sodium-dicarboxylate cotransporter (NaDC) and Na^+ gradient is, in turn, driven by Na^+-K^+-ATPase (Pritchard, 1987; Pritchard, 1995; Welborn et al., 1998).

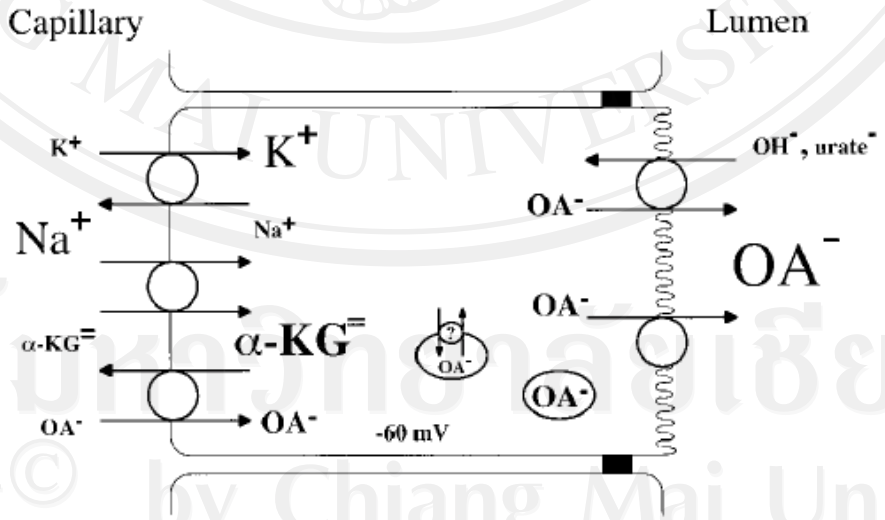

Figure 5 The classical model of the mechanism of basolateral organic anion transport system (Sweet et al., 2001).

Regulation of renal organic anion transporters

The regulation of Oats activities have been studied and classified to long- and short-term regulations. Long-term or chronic regulation of Oats have been demonstrated at transcriptional and translational processes within a time frame of hours to days whereas the short-term regulation could show in hours or less (Duan and You, 2010). In addition, long-term regulation has been shown when the body undergoes massive changes, such as during growth development or the occurrence of particular diseases (Buist, 2002; Naud et al., 2008; Sakurai et al., 2005). Hence, there were several factors involved in the regulation of Oats, including hormones, intracellular proteins, nuclear receptors, scaffolding proteins, and diseases (Duan et al., 2010; Li et al., 2010).

Recently, the regulatory mechanisms of organic anion transport have been extensively identified. As shown in Figure 6, the stimulatory effect of epidermal growth factor (EGF) on Oat3 expression and function has been proposed (Sauvant et al., 2001, 2002, 2003; Soodvilai et al., 2004).

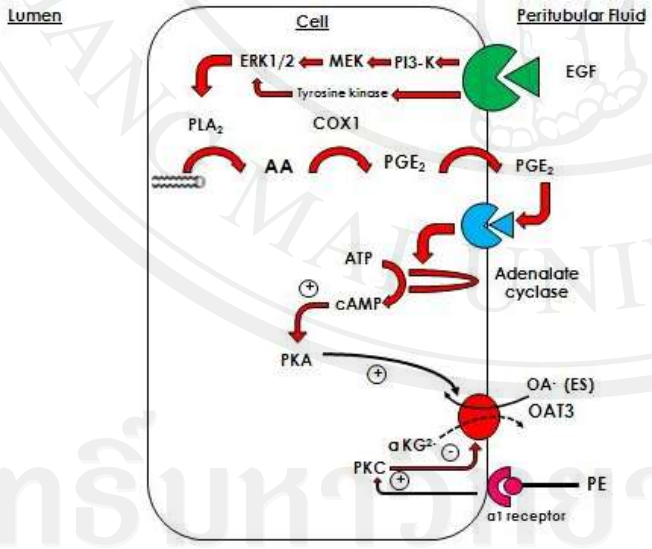

Figure 6 Modified predicted model for epidermal growth factor (EGF) stimulation on organic anion transporter 3 expression and function (Soodvilai et al., 2004; Soodvilai et al., 2005)

Briefly, EGF bound to its receptor in renal proximal tubular cells, leading to the activation of phospho-inositide-3-kinase (PI3K), mitogen-activated protein kinase (MEK), extracellular signal regulated kinase (ERK1/2), and phospholipase A2

(PLA2), respectively. These consequences led to an increase in the production of arachidonic acid (AA), which was metabolized to PGE2 via cyclooxygenase I (COX1). Subsequently, PGE2 activated the production of cAMP, resulting in the activation of PKA and final stimulation of Oat3 function and expression. On the other hand, various studies demonstrated that the function and expression of Oats could be regulated by the activation or inhibition of PKC, depending on PKC isoforms. For example, angiotensin II inhibited hOAT3 activity through the activation of PKCα (Duan et al., 2010) while bradykinin increased hOAT3 expression at proximal tubular cell surface via PKCζ, PKCε and PKCδ activation (Duan et al., 2010; Li et al., 2010). Moreover, it has been shown that insulin, as well as EGF, stimulated rat and mouse Oat3 (rOat3 and mOat3) transport expressions and functions through PKCζ activation. (Barros et al., 2009)