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Review

3-Deoxyglucosone: metabolism, analysis, biological activity, and clinical implication

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Abstract

3-Deoxyglucosone (3-DG) is synthesized via the Maillard reaction and the polyol pathway, and is detoxified to 3-deoxyfructose and 2-keto-3-deoxygluconic acid. 3-DG rapidly reacts with protein amino groups to form advanced glycation end products (AGEs) such as imidazolone, pyrraline, N^{ε}-(carboxymethyl)1ysine and pentosidine, among which imidazolone is the AGE most specific for 3-DG. As demonstrated by using gas chromatography–mass spectrometry or high-performance liquid chromatography, plasma 3-DG levels are markedly increased in diabetes and uremia. Although the plasma 3-DG levels had been controversial, it was clearly demonstrated that its plasma level depends on the deproteinization method by which either free or total 3-DG, presumably bound to proteins, is measured. In diabetes, hyperglycemia enhances the synthesis of 3-DG via the Maillard reaction and the polyol pathway, and thereby leads to its high plasma and erythrocyte levels. In uremia, however, the decreased catabolism of 3-DG, which may be due to the loss of 3-DG reductase activity in the end-stage kidneys, may lead to high plasma 3-DG level. The elevated 3-DG levels in plasma and erythrocytes may promote the formation of AGEs such as imidazolone, as demonstrated by immunohistochemistry and immunochemistry using an anti-imidazolone antibody. Although AGE-modified proteins prepared in vitro exhibit a variety of biological activities, known AGE structures have not yet been demonstrated to show any biological activities. Because 3-DG is potent in the formation of AGEs and has some biological activities, such as cellular toxicity, it may be more important in the development of diabetic and uremic complications than the known AGE structures. By demonstrating that treatment with an aldose reductase inhibitor reduces the erythrocyte levels of 3-DG and AGEs, such as imidazolone, light is shed on the mystery of how aldose reductase inhibitors may prove beneficial in diabetic complications. These evidences suggest that 3-DG plays a principal role in the development of diabetic and uremic complications.

Keywords: Reviews; 3-Deoxyglucosone

Contents

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1. Introduction

Non-enzymatic glycation of proteins has drawn the attention of clinical researchers since the discovery of HbA_{1c} as a marker of hyperglycemia in diabetic patients. The occurrence of early products of the non-enzymatic sugar–protein interaction result from the covalent addition of the reducing sugars to protein amino groups. In the intermediate and late stages of non-enzymatic reaction of glucose with proteins, the spontaneous formation of highly reactive compounds, such as 3-deoxyglucosone (3-DG) and terminal products, called collectively advanced glycation end products (AGEs), may account for the numerous features of diabetic complications as well as aging. Our group has also suggested that 3-DG is responsible for the development of uremic complications. This article reviews the methods for quantification of 3-DG in body fluids, because its plasma levels in humans have been controversial. Further, in this review, recent studies are highlighted which indicate that 3-DG plays an important role in aging and the development of not only diabetic but also uremic complications by exerting its potent ability to form AGEs and the other biological activities.

2. Metabolism of 3-deoxyglucosone (3-DG)

2.1. The Maillard reaction

Glucose reacts non-enzymatically with protein amino groups to initiate glycation, the early stage of the Maillard reaction. This process begins with the conversion of reversible Schiff base adducts to stable, covalently bound Amadori rearrangement products. The levels of the Amadori products on numerous proteins are elevated in proportion to the degree of hyperglycemia in diabetes mellitus. In the intermediate stage of the Maillard reaction, the Amadori products can then undergo multiple dehydration and rearrangements to produce highly reactive carbonyl compounds such as 3-DG [1–4] (Fig. 1), which reacts again with free amino groups, leading to crosslinking and browning of the proteins via the formation of AGEs in the late stage of the Maillard reaction [5].

Zyzak et al. [6] studied the decomposition of the model Amadori compound, N^{α}-formyl-N^{ε}-fructoselysine and of Amadori compounds on glycated collagen under physiological conditions. Glucose and mannose were identified as major products formed by reversal of the Amadori rearrangement, along with tetroses, pentoses, and 3-DG, formed by reverse aldol, rearrangement, and hydrolysis reactions. Thus, the spontaneous decomposition of Amadori compounds to more reactive sugars in vivo, including 3-DG, provides a mechanism for propagating damage to proteins as a result of glycation of proteins by glucose in vivo.

Hayase et al. [7] demonstrated that the generation of fluorescence, browning, and polymerization, as well as the formation of 3-DG, are accelerated under non-aerobic, compared to aerobic, conditions in the incubation of lysozyme with glucose. Under non-aerobic conditions, the formation of AGEs from

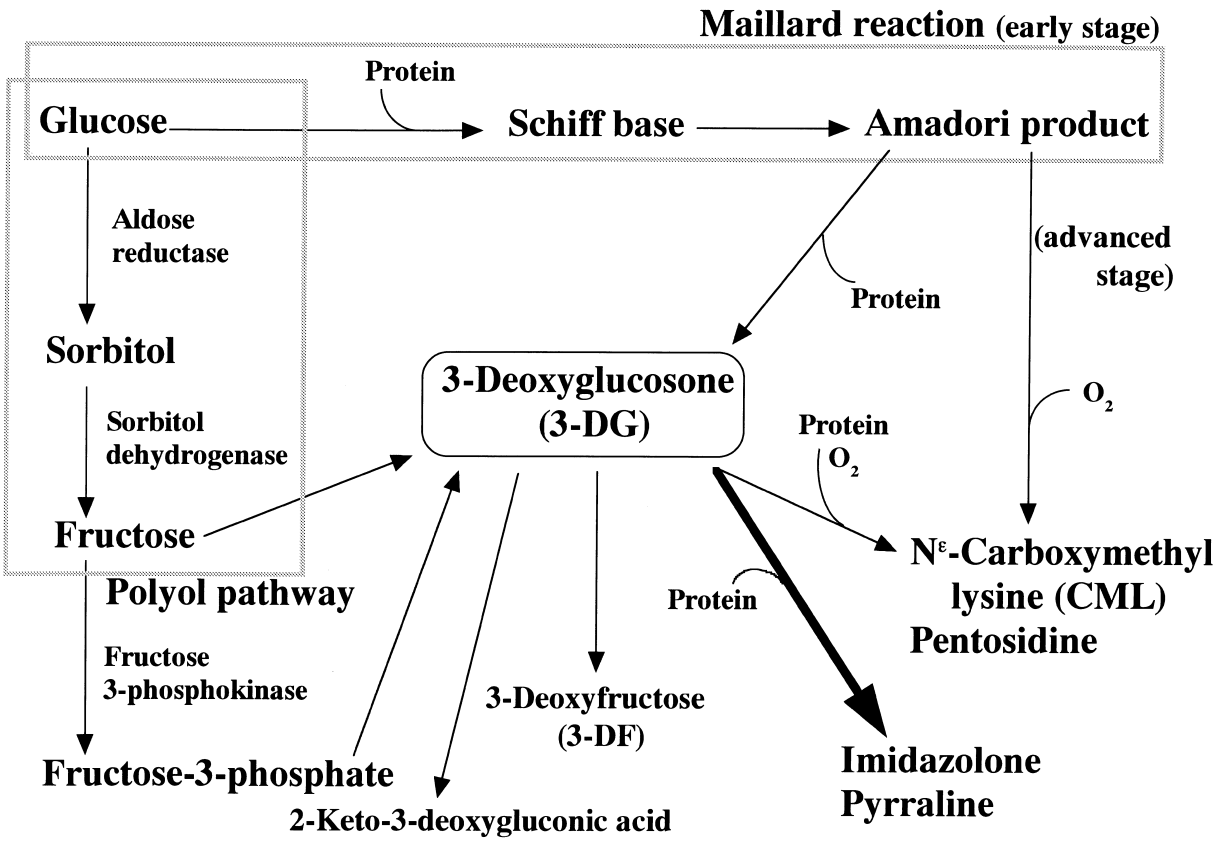

Fig. 1. Synthesis of 3-deoxyglucosone (3-DG) and advanced glycation end products (AGEs) via the Maillard reaction and the polyol pathway, and enzymatic reduction of 3-DG to 3-deoxyfructose (3-DF).

3-DG via Amadori compounds is the major pathway, because the formation of CML, glyoxal, and glucosone is accelerated by an oxidative reaction catalyzed with transition metal ions.

Several compounds such as CML [8], pyrraline [9], pentosidine [10], crosslines [11] and imidazolone [12,13] have been proposed as candidates for the structures of AGEs (Fig. 2). CML is an AGE formed on protein by combined non-enzymatic glycation and oxidation (glycoxidation) reactions, but is also formed during metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of protein [14]. Thus, both glycoxidation and lipid peroxidation may be important sources of CML in tissue proteins in vivo, and CML may be a general marker of oxidative stress and long-term damage to protein in aging, atherosclerosis, diabetes and dialysis-related amyloidosis. Pentosidine is also produced as a glycoxidation product. Although these AGEs and glycoxidation products are implicated in the development of diabetic complications, these compounds are present at only trace concentrations in tissue proteins and account for only a fraction of the chemical modifications in AGE-proteins prepared in vitro [15].

Pyrraline is an AGE formed by non-enzymatic reaction initiated by glucose with lysine residues on proteins. This reaction involves 3-DG as an intermediate. Bovine lens α-crystallins incubated with 3-DG showed that pyrraline formation was a major modification [16]. Portero et al. [17] demonstrated time- and sugar concentration-dependent increase in pyrraline formation in serum albumin incubated with either glucose or 3-DG. Formation of pyrraline from 3-DG was rapid at slightly acidic pH, confirming its

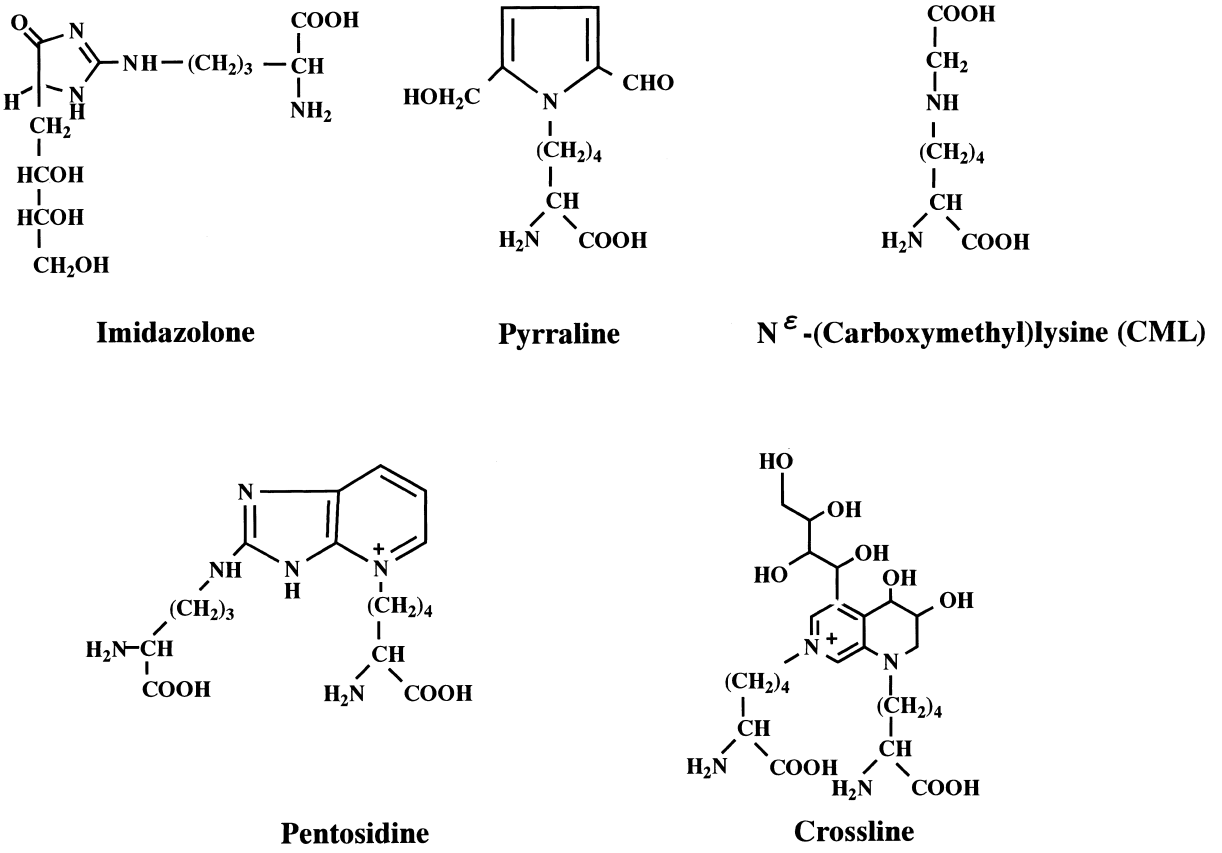

Fig. 2. Chemical structures of known AGEs, such as imidazolone, pyrraline, N^{ε}-(carboxymethyl)lysine (CML), pentosidine and crossline.

synthetic pathway through this Maillard reaction intermediate. They could detect pyrraline in human skin collagen and human plasma.

Recently, imidazolone was isolated as a novel AGE in vitro from the incubation mixture of 3-DG and an arginine derivative [12,13]. 3-DG first attacks arginine residues in proteins and then reacts with the lysine residues under physiological conditions [1,2]. Thus, 3-DG reacts with guanidino groups of arginine residues in the proteins, forming imidazolone modification of the arginine residues (Fig. 3). The formation of imidazolone by incubating 3-DG with arginine is very rapid, reaching a maximum concentration within 24 h [18].

Incubation of 3-DG with proteins also leads to the formation of pyrraline [19], pentosidine [20], CML [21,22] as well as imidazolones [12,13]. Among these AGEs, imidazolone is the AGE most specific for the in vivo involvement of 3-DG in the modification of tissue proteins. We first detected imidazolone in vivo in $\beta_{2\text{-microglobulin}}$-amyloid deposits obtained from hemodialysis patients [23], and also in the kidneys and aortas of diabetic patients [24], by immunohistochemistry using a monoclonal anti-imidazolone antibody (AG-1).

The modification of long-lived proteins with AGEs has been hypothesized to contribute to the development of pathologies associated with diabetes mellitus [24–29], aging [30], dialysis-related amyloidosis [21–23,31,32] and Alzheimer disease [33–35]. The in vivo presence of AGEs has been demonstrated in hemoglobin [28,29], lens crystallin

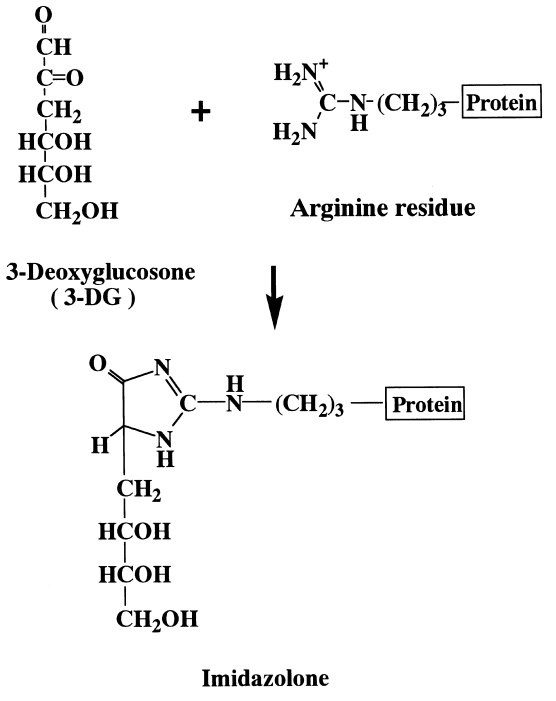

Fig. 3. Non-enzymatic synthesis of imidazolone by reacting 3-DG with arginine residues of proteins.

[30,36], $\beta_{2\text{-microglobulin}}$ [21–23,31,32], β-amyloid peptide and τ protein [33–35]. It has also been shown that AGE-modified peptides accumulate in the circulation of diabetic and non-diabetic patients with uremia [37]. Hb-AGE may prove superior to HbA_{1c} as a long-term index of circulating glucose concentrations [28].

2.2. Polyol pathway

Polyol pathway may be associated with production of 3-DG. 3-DG was demonstrated to be non-enzymatically formed in the absence of amino groups from fructose, an oxidized product of sorbitol by sorbitol dehydrogenase in the polyol pathway [38]. 3-DG has also been reported to be a biophysical hydrolysis product of fructose-3-phosphate, which was identified in the lens and hearts of diabetic rats [39–42]. Fructose-3-phosphate is considered to be enzymatically produced from fructose [41,43]. These data suggest that the formation of 3-DG may occur via the polyol pathway in vivo (Fig. 1), and be involved in the development of diabetic complications.

Lal et al. [42] could detect fructose-3-phosphate using ^{31}P nuclear magnetic resonance (NMR) in the extracts of the hearts from diabetic rats but not from normal rats. By using GC–MS they could also detect 3-DG, a decomposition product of fructose-3-phosphate, at a concentration approximately 10 times higher than in controls. However, they could not detect 3-deoxyfructose and 2-keto-3-deoxygluconic acid, detoxication products of 3-DG. Fructose and sorbitol levels were also elevated in the hearts of diabetic rats. Unexpectedly, an aldose reductase inhibitor (sorbinil) treatment had no effect on the levels of these metabolites. Their data suggest that either the heart may be unique in its production of fructose or it may not readily transport the aldose reductase inhibitor. Production of the potent glycating agents, fructose-3-phosphate and 3-DG, in diabetics suggests that these compounds may be contributing factors in the glycation of cardiac proteins in the diabetic rat heart.

Hamada et al. [44] studied the effect of treatment with epalrestat, an aldose reductase inhibitor, on the concentration of fructose-3-phosphate and AGEs in erythrocytes from diabetic patients. The diabetic patients showed elevated erythrocyte levels of fructose-3-phosphate and AGEs. The aldose reductase inhibitor treatment decreased their erythrocyte levels. They also demonstrated that incubation of albumin with fructose-3-phosphate or 3-DG resulted in a greater production of AGEs compared to incubation with glucose or fructose.

We demonstrated that erythrocyte 3-DG is produced via the polyol pathway in diabetic hemodialysis patients [45]. The erythrocyte levels of 3-DG were measured in the patients before and after aldose reductase inhibitor (epalrestat) treatment. The erythrocyte levels of sorbitol, 3-DG, and AGEs, such as imidazolone and CML, were significantly elevated in diabetic hemodialysis patients as compared with healthy subjects. The administration of the aldose reductase inhibitor significantly decreased the erythrocyte levels of sorbitol, 3-DG and imidazolone, and tended to decrease the CML level. Thus, AGEs as

well as 3-DG in the erythrocytes of these patients are produced mainly via the polyol pathway. The aldose reductase inhibitor may prevent diabetic and uremic complications associated with AGEs.

2.3. Catabolism

A major urinary metabolite of 3-DG administered to rats was identified as 3-deoxyfructose [46]. 3-Deoxyfructose has also been detected in human urine and plasma [15,47]. Thus, 3-DG is detoxified by the reducing enzyme to 3-deoxyfructose, which is then excreted to urine. The ability to detoxify 3-DG by the enzyme may provide a genetic basis for differences in the severity of age-related pathologies and diabetic complications. The elevated serum levels of 3-DG in diabetic patients may be due to the increased production of 3-DG via the Maillard reaction resulting from hyperglycemia. However, the reason why the serum 3-DG level in uremic patients is increased, is not clear at present. Because 3-DG is not excreted into urine in healthy subjects [47], the reduced renal clearance of 3-DG by the diseased kidneys cannot explain the elevated serum 3-DG level in uremic patients. The activity of 3-DG-reducing enzymes which convert 3-DG to 3-deoxyfructose, may be suppressed in uremia, leading to the accumulation of 3-DG in uremic serum.

Sato et al. [48] reported that NADPH-dependent 3-DG-reducing enzyme activity was detected in the extracts of various monkey tissues, among which kidney exhibited the highest specific activity. They purified a dimeric enzyme identical to dimeric dihydrodiol dehydrogenase, and two monomeric enzymes similar to human liver aldehyde reductase and human placental aldose reductase from monkey kidneys. They also reported that dimeric dihydrodiol dehydrogenase detected in dog liver cytosol oxidized *trans*-cyclohexanediol, and *trans*-dihydrodiols of benzene and naphthalene, and in the reverse reaction in the presence of NADPH as a coenzyme, it reduced α-dicarbonyl compounds, such as 3-DG [49]. The dimeric enzyme was detected in the liver and kidneys of dogs, and was immunochemically similar to the dimeric enzymes from monkey kidneys, rabbit lens, and pig liver.

Kanazu et al. [50] reported that 3-DG reductase activity in the extracts of rat, pig and human livers was potently inhibited by aldehyde reductase inhibitors, and that the major species of 3-DG reductase purified from human and pig livers were identical with aldehyde reductase. Takahashi et al. [51] demonstrated that a 3-DG-reducing enzyme is identical with aldehyde reductase. A variety of rat tissues showed fairly high levels of gene expression of aldehyde reductase. This suggests that sufficient aldehyde reductase is present to detoxify 3-DG when it is formed through the Maillard reaction. The enzyme was found to be partially glycated at lysines 67, 84, and 140, and glycation of the enzyme was prominent in the kidneys of streptozotocin-induced diabetic rats [52]. The glycated enzyme had low catalytic efficiency as compared to the nonglycated form. Since the enzyme plays a role in detoxifying 3-DG, glycation of aldehyde reductase with reduction of its activity may lead to elevation of 3-DG and enhanced formation of AGEs in diabetes.

Matsuura et al. [53] demonstrated that 3-DG reductase purified from dog adrenal glands is identical with aldose reductase. Adrenal glands showed the highest NADPH-linked 3-DG reductase activity of dog tissues.

In addition, 3-DG is detoxified to 2-keto-3-deoxygluconic acid by oxoaldehyde dehydrogenase as demonstrated by Fujii et al. [54], who demonstrated that 3-DG was easily taken into the erythrocytes, and was converted to 2-keto-3-deoxygluconic acid. Thus, oxoaldehyde dehydrogenase may also play an important role in the prevention of AGE formation from 3-DG.

3. Analysis of 3-DG

3.1. Gas chromatography–mass spectrometry (GC–MS)

Knecht et al. [47] developed a GC–MS method for measuring 3-DG and 3-deoxyfructose in human urine and plasma (Table 1). 3-Deoxyfructose is a less reactive metabolite derived from enzymatic reduction of 3-DG, and is excreted into urine. 3-DG and 3-deoxyfructose were reduced to 3-deoxyhexitol, using either $NaBH_4$ or $NaBD_4$, and then analyzed by selected ion monitoring (SIM)-GC–MS. Based on comparative analysis of samples reduced with

Table 1
Mean plasma or serum concentrations of 3-DG reported previously[a]

References	Sample	3-DG (ng/ml)			Method
		Normal	DM	HD	Equipment (detection); deproteinization; I.S.; sample treatment; and derivatization
Knecht et al. [47]	Human plasma	10			GC–MS (SIM); TCA [1,2-^{2}H]3-DH; reduction of 3-DG & 3-DF to 3-DH, per-*O*-acetylation
Niwa et al. [55]	Human serum	314	778		GC–MS (SIM); ethanol; [$^{13}C_6$]3-DG; MO-TMS derivatization of 3-DG
Yamada et al. [59]	Rat plasma	61.4	149		HPLC (Fl); perchloric acid; 3,4-hexanedione; conjugation of 3-DG with 2,3-diaminonaphthalene
Niwa et al. [22]	Human serum	270	694	1484	GC–MS (SIM); ethanol; [$^{13}C_6$]3-DG; MO-TMS derivatization of 3-DG
Hamada et al. [60]	Human plasma	12.8	31.8		HPLC (Fl); perchloric acid; 3,4-hexanedione; conjugation of 3-DG with 2,3-diaminonaphthalene
Lal et al. [58]	Human plasma	9.5	16		GC–MS (SIM); ultrafiltration; [$^{13}C_6$]3-DG; conjugation of 3-DG with 2,3-diaminonaphthalene, TMS derivatization
	Human plasma	277			GC–MS (SIM); ethanol; [$^{13}C_6$]3-DG; conjugation of 3-DG with 2,3-diaminonaphthalene, TMS derivatization

[a] 3-DG, 3-deoxyglucosone; DM, diabetes mellitus; HD, hemodialysis; I.S., internal standard; TCA, trichloroacetic acid; GC–MS, gas chromatography–mass spectrometry; SIM, selected ion monitoring; Fl, fluorescence; 3-DH, 3-deoxyhexitol; 3-DF, 3-deoxyfructose; HPLC, high-performance liquid chromatography; MO-TMS, methoxime-trimethylsilyl.

$NaBH_4$ versus $NaBD_4$, 3-deoxyhexitol in urine was derived exclusively (greater than 99%) from 3-deoxyfructose, while 3-DG accounted for approximately 15% of 3-deoxyhexitol in plasma. Their data indicate that several milligrams of 3-DG are formed in the body per day and detoxified by reduction to 3-deoxyfructose. However, this method does not directly measure plasma 3-DG, but indirectly measures it after reduction of 3-DG to 3-deoxyhexitol.

We have developed a GC–MS method for direct quantification of 3-DG in serum [22,55–57] and erythrocytes [45] (Table 1). Briefly, after adding [$^{13}C_6$]3-DG as an internal standard into serum, the sample was incubated with glucose oxidase, catalase and mutarotase in phosphate buffer. The mixture was deproteinized by addition of ethanol and subsequent centrifugation. The supernatant was applied to a cation exchange column and eluted with distilled water. The collected eluate was then applied to an anion exchange column, and eluted with distilled water. The eluate was collected and lyophilized. Carbonyl groups of the residue were transformed to the methoxime derivatives with methoxylamine hydrochloride. After evaporation over a nitrogen stream, hydroxyl groups were transformed to the trimethylsilyl derivatives. The sample was subjected to SIM-GC–MS using isobutane as a reactant gas for chemical ionization (CI).

We first measured serum 3-DG levels in diabetic and uremic patients using GC–MS, and demonstrated that their serum levels are increased in these patients [22,55–57] (Fig. 4). The protonated molecule ions, $(M+H)^+$, of 3-DG and [$^{13}C_6$]3-DG showed base peaks at m/z 437 and m/z 443, respectively. Methoximation forms *syn* and *anti* products due to a restricted rotation around the methoxime double bond, so that four isomers can be produced due to the presence of two carbonyl groups in 3-DG. However, in view of thermodynamic stability, the methoxime-trimethylsilyl derivative of 3-DG showed only two peaks by GC–MS. The presence of 3-DG in normal and uremic serum was also confirmed by electron ionization (EI)-SIM, which monitored the base peak ions of 3-DG and [$^{13}C_6$]3-DG at m/z 231 and 235, respectively.

A significant discrepancy (over 30-fold) exists in the reported values of plasma 3-DG. Knecht et al. [47] reported the levels of plasma 3-DG in normoglycemics to be 10 ng/ml, using a GC–MS procedure. In contrast to this, we reported 3-DG levels to be 314 ng/ml in normoglycemics, using a totally independent GC–MS method [55] (Table 1). To resolve this disagreement, Lal et al. [58] devised a GC–MS procedure for its measurement. Plasma samples were deproteinized either by ultrafiltration, or by addition of ethanol as described by us [55]. 3-DG in the ultrafiltrate or the supernatant was conjugated with 2,3-diaminonaphthalene to produce a stable adduct which was then converted to a silyl ether and analyzed by SIM-GC–MS using an internal standard of [$^{13}C_6$]3-DG. Using this approach, mean 3-DG levels in plasma deproteinized by ultrafiltration were elevated from 9.5 ng/ml in normoglycemics to 16 ng/ml in diabetics. When deproteinization of the plasma was carried out using ethanol, the levels of 3-DG from normoglycemic

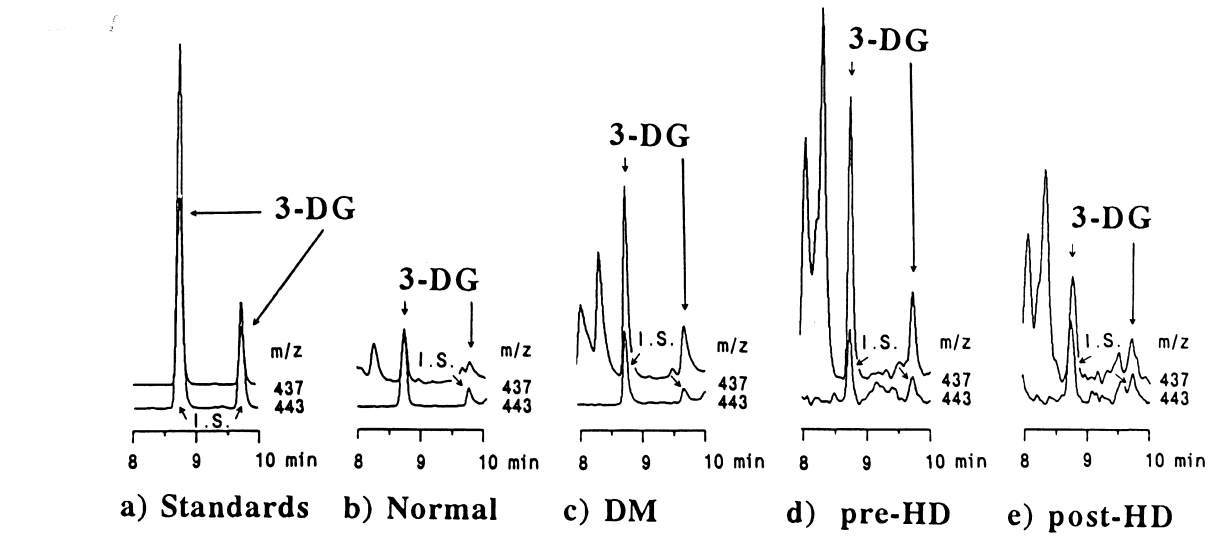

Fig. 4. Chemical ionization-selected ion monitoring (CI-SIM) chromatograms of 3-DG and [$^{13}C_6$]3-DG (an internal standard) in a standard sample (a) and serum samples from a normal subject (b), a diabetic patient (DM) (c), and a hemodialysis patient before (pre-HD) (d) and after hemodialysis (post-HD) (e).

plasma were similar to those reported by us (277 ng/ml) (Table 1). Their results suggest that 3-DG levels measured by ultrafiltration may represent free circulating 3-DG, and those obtained by ethanol extraction may represent a form of total 3-DG bound to a macromolecule, presumably protein.

3.2. High-performance liquid chromatography (HPLC)

Yamada et al. [59] developed an HPLC method for measuring plasma 3-DG, and demonstrated that plasma 3-DG levels were elevated in streptozotocin-induced diabetic rats than in normal rats (Table 1). 3-DG was converted by reacting with 2,3-diaminonaphthalene to a stable compound, 2-(2,3,4-trihydroxybutyl)-benzo[*g*]quinoxaline, which was detected at UV (268 nm) or fluorescence (excitation, 271 nm; emission, 503 nm) by HPLC. Plasma 3-DG levels were suppressed by administering aminoguanidine, an inhibitor of the Maillard reaction. Plasma pyrraline levels in diabetic rats increased in parallel with elevated 3-DG levels, but were only marginally suppressed by administration of aminoguanidine. Their quantification method used 3,4-hexanedione as an internal standard. The conjugation of 3-DG with 2,3-diaminonaphthalene was done at 4°C overnight. However, because 3-DG reacts very rapidly with amino groups such as arginine even for 1 h to form imidazolone [18], some amounts of 3-DG will not conjugate with 2,3-diaminonaphthalene, but react with the amino groups such as arginine to form the other derivatives such as imidazolone. Thus, the plasma 3-DG levels might be estimated at apparently lower concentrations. If a stable isotope of 3-DG is used as an internal standard, the loss of 3-DG during the conjugation reaction will be counterbalanced as the ratio of 3-DG to stable isotope-labelled 3-DG.

According to the method developed by Yamada et al. [59], Hamada et al. [60] quantitated plasma 3-DG levels in diabetic patients (Table 1). They also demonstrated that plasma 3-DG was significantly increased in diabetic patients compared to non-diabetic control subjects. 3-DG levels were well

correlated with plasma glucose and HbA_{1c} levels in diabetic patients. The improvement of hyperglycemia resulted in a significant decrease in 3-DG. Thus, plasma glucose level is a predominant factor that determines the plasma 3-DG levels in diabetic patients.

Fujii et al. [61] developed an alternate HPLC method to measure 3-DG. 3-DG was oxidized with crude oxoaldehyde dehydrogenase to 2-keto-3-deoxygluconic acid, which was derivatized with 1,2-diamino-4,5-methylenedioxybenzene, and the fluorescent products were detected and quantitated by HPLC using a solvent containing borate. Oxoaldehyde dehydrogenase was prepared from rabbit liver and partially characterized. This method has been used to measure 3-DG levels in the incubation solutions of bovine serum albumin with glucose in the presence or absence of aminoguanidine. However, this method has not yet been applied to the measurement of plasma 3-DG levels.

By using this method, Fujii et al. [54] demonstrated that considerable amounts of 2-keto-3-deoxygluconic acid are present in both human erythrocytes and plasma. Human erythrocytes contained about 30–50 times higher 2-keto-3-deoxygluconic acid than human plasma did, and also had the same ability to convert 3-DG to 2-keto-3-deoxygluconic acid as oxoaldehyde dehydrogenase did. The erythrocyte levels of 2-keto-3-deoxygluconic acid in diabetic patients were higher than in healthy subjects. When erythrocytes were incubated with 3-DG, 3-DG was easily taken into the erythrocytes, and was converted to 2-keto-3-deoxygluconic acid. Thus, oxoaldehyde dehydrogenase may play an important role in the prevention of glycation of not only hemoglobin but also blood vessels by scavenging plasma 3-DG into erythrocytes.

4. Biological activity of 3-DG

4.1. Induction of heparin-binding epidermal growth factor-like growth factor

3-DG selectively induced heparin-binding epidermal growth factor (HB-EGF)-like growth factor mRNA in rat aortic smooth muscle cells, and increased the secretion of HB-EGF from rat aortic smooth muscle cells [62]. 3-DG augmented intracellular peroxides prior to the induction of HB-EGF mRNA. *N*-Acetyl-L-cysteine and aminoguanidine suppressed 3-DG-induced HB-EGF mRNA and intracellular peroxide levels in rat aortic smooth muscle cells. 3-DG induces HB-EGF by increasing the intracellular peroxide levels. Since HB-EGF is known as a potent mitogen for smooth muscle cells and is abundant in atherosclerotic plaques, the induction of HB-EGF by 3-DG with the simultaneous elevation of intracellular peroxides may trigger atherogenesis in uremic patients as well as diabetic patients.

4.2. Induction of apoptosis

3-DG induced apoptosis of monocytic leukemia cells at physiological concentrations [63]. Both ladder formation of DNA and nuclear fragmentation were observed in the 3-DG-treated cells, indicating that apoptotic cell death was induced. The fluorescent intensity of an oxidation-sensitive dye was increased in the cells. Apoptosis and intracellular oxidant levels were enhanced by an inhibitor of glutathione biosynthesis, and partially blocked by *N*-acetyl-L-cysteine, an antioxidant. Thus, intracellular oxidant stress is a cause of the apoptosis induced by 3-DG.

The gene of aldehyde reductase which catalyzes the reduction of 3-DG, was overexpressed in rat pheochromocytoma PC12 cells [64]. Cytotoxicity of 3-DG to induce apoptotic cell death was determined by fluorescent microscopy. In the aldehyde reductase gene-transfected cells, the cytotoxicity of 3-DG and apoptotic cell death were decreased. Thus, the intracellular aldehyde reductase protects neural cells from the cytotoxicity of 3-DG, and that neural cells, which normally express a low level of aldehyde reductase, might be susceptible to diabetic complications caused by 3-DG.

4.3. Suppression of cell proliferation

3-DG suppressed the proliferation of various cell lines by inhibition of DNA synthesis. The cells proliferating actively, in which the intracellular GSH concentration have been reported to be lower, were

more susceptible to the inhibitory effects of 3-DG on the cell-cycle progression during the S phase [65].

4.4. Inactivation of glutathione reductase

Vander et al. [66] demonstrated that 3-DG, and the other reactive endogenous aldehydes, such as methylglyoxal and xylosone inactivated glutathione reductase, a central antioxidant enzyme. However, these 2-oxoaldehydes were much less effective than 4-hydroxynonenal, which is a product of oxidative degradation of unsaturated lipids, and is an endogenous reactive α,β-unsaturated aldehyde with numerous biological activities. In addition to 4-hydroxynonenal, 3-DG may stimulate a positive feedback loop by inactivating glutathione reductase that enhances the potential for oxidative damage.

4.5. Suppression of enzyme activities of glucose metabolism

3-DG inhibited the activities of mouse hepatic enzymes responsible for glucose metabolism [67]. 3-DG markedly inhibited hexokinase and glucose-6-phosphate dehydrogenase activities, while it scarcely affected glucokinase, glucose-6-phosphatase, and phosphofructokinase activities. Thus, 3-DG inhibits the intake of glucose in the liver, and may be involved in the aggravation of hyperglycemia in diabetes.

5. Clinical implication of 3-DG

5.1. Aging

Lal et al. [40] observed an age-associated increase in the concentrations of polyol pathway-associated metabolites, such as sorbitol, fructose, sorbitol-3-phosphate and fructose-3-phosphate, in the lens, although the lenticular glucose levels were not elevated. Because fructose-3-phosphate is a potent glycating agent and a potential in vivo source of 3-DG, its accumulation in the lens, along with fructose, may be a contributing factor in the age-associated increase of non-enzymatic glycation in rat lenses.

Pyrraline is an AGE formed by 3-DG and lysine residues on proteins [68]. Nagaraj and Sady [16] demonstrated that pyrraline concentration in cataractous lenses was higher than in age-matched normal lenses. Unexpectedly, in diabetic lenses, pyrraline concentration was lower than normal lenses. Thus, the formation of AGEs such as pyrraline occurs in the human lens and such modifications may play a role in lens aging and cataract formation.

5.2. Diabetes mellitus

We first demonstrated that serum concentration of 3-DG is elevated in diabetic patients as compared with healthy subjects [55]. More notably, the serum concentration of 3-DG is higher in diabetic patients with nephropathy than those without nephropathy. This result suggests that 3-DG may be responsible for the development of diabetic nephropathy by promoting the formation of AGEs. In diabetic patients, hyperglycemia may increase the production of 3-DG mainly through the Amadori products to proteins, leading to increased serum levels of 3-DG.

We also demonstrated that serum levels of 3-DG and AGE (imidazolone) contents in the kidney and the lens were significantly higher in streptozotocin (STZ)-induced diabetic rats with nephropathy than those in control rats [18,57]. Thus, high serum levels of 3-DG may result in the occurrence of diabetic complications such as nephropathy and cataract.

We measured erythrocyte levels of imidazolone in diabetic patients using enzyme-linked immunosorbent assay (ELISA) with a monoclonal anti-imidazolone antibody (AG-1) [24]. The imidazolone levels in the erythrocytes of diabetic patients were found to be significantly increased as compared with those of healthy subjects. Immunohistochemistry using the antibody demonstrated that imidazolone was detected in nodular lesions and expanded mesangial matrix of glomeruli, and renal arteries in an advanced stage of diabetic nephropathy, as well as in atherosclerotic lesions of aortas. These results, taken together with a recent demonstration of increased serum 3-DG levels in diabetes, strongly suggest that imidazolone produced by 3-DG may contribute to the progression of long-term diabetic complications such as nephropathy and atherosclerosis.

Lal et al. [41] detected elevated concentrations of

3-DG along with its detoxification product, 3-deoxyfructose, in diabetic rat lense. Fructose-3-phosphate and sorbitol-3-phosphate are produced in diabetic rat lenses by 3-phosphokinase. While sorbitol-3-phosphate appears to be an inert polyol phosphate, fructose-3-phosphate is a potent cross-linking agent and a potential in vivo source of 3-DG. They suggested that fructose-3-phosphate and 3-DG are likely to be important contributors to the process of non-enzymatic glycation in diabetic rat lenses.

Szwergold et al. [39] identified fructose-3-phosphate in the lens of diabetic rats, but not in normal lens. Fructose-3-phosphate is relatively labile and undergoes hydrolysis to yield inorganic phosphate and the potent glycosylating agent, 3-DG. The increase in the concentration of fructose-3-phosphate in the lens of diabetic rats suggests that it and its hydrolysis product, 3-DG, may be responsible in part for the development of some diabetic complications in the lens.

Wells et al. [15] detected 3-deoxyfructose, a metabolite of 3-DG, in plasma and urine of diabetic patients at higher concentrations compared with healthy subjects. Plasma and urinary 3-deoxyfructose concentrations correlated strongly with one another, with HbA_{1c}, and with urinary N^{ε}-fructoselysine. The overall increase in 3-deoxyfructose concentrations in plasma and urine in diabetes and their correlation with the other indexes of glycemic control suggest that increased amounts of 3-DG are formed in the body during hyperglycemia in diabetes and then metabolized to 3-deoxyfructose.

Portero et al. [17] detected pyrraline-like material in human plasma proteins following enzyme digestion and analysis by HPLC. Plasma from diabetic patients showed a significant increase in pyrraline-like material compared to controls, reflecting thereby the elevated levels of the immediate precursor of pyrraline, 3-DG, in diabetic plasma.

5.3. Uremia

Serum 3-DG levels are elevated not only in diabetic patients [55] but also in uremic patients [22,56]. As shown in Fig. 5, serum levels of 3-DG are increased in hemodialysis, continuous ambulatory peritoneal dialysis (CAPD) and undialyzed uremic patients as compared to normal subjects and diabetic patients [22]. The serum levels of 3-DG decrease after hemodialysis with a mean reduction rate of 67%, because 3-DG is a small molecule with a molecular weight of 162. Even after hemodialysis, however, the serum levels of 3-DG are significantly higher than those in normal subjects. The serum levels of 3-DG in the undialyzed uremic, hemodialysis and CAPD patients are high even compared to the diabetic patients. In undialyzed uremic patients serum 3-DG is positively correlated with serum creatinine.

In monkey, the total activity of 3-DG-reducing enzymes that were composed of aldehyde reductase, aldose reductase and dihydrodiol dehydrogenase, was most abundant in the kidneys among the various tissues [48]. In uremic patients, the activity of 3-DG-reducing enzymes in the kidneys will be lost due to organization of the end-stage kidneys, and consequently 3-DG is not rapidly metabolized to less reactive 3-deoxyfructose, causing the accumulation of 3-DG in uremic serum. Our results demonstrate that the serum levels of 3-DG are much higher in the uremic patients than in diabetic patients.

We demonstrated that the erythrocyte levels of sorbitol, 3-DG, imidazolone, and CML were significantly elevated in diabetic hemodialysis patients compared with healthy subjects [45]. The administration of an aldose reductase inhibitor (epalrestat) significantly decreased the erythrocyte levels of sorbitol, 3-DG, and imidazolone, and tended to decrease the CML level. Thus, aldose reductase inhibitor treatment may prevent diabetic and uremic complications associated with AGEs.

Münch et al. [69] developed two competitive ELISAs for measurement of serum AGEs, using a monoclonal antibody directed against imidazolone, an AGE formed by the reaction of arginine with 3-DG, and a polyclonal antibody directed against keyhole limpet hemocyanin-AGE. Each of the assays showed significant elevation of serum AGE levels in hemodialysis patients compared with healthy subjects. These methods will be particularly valuable for monitoring the removal of AGEs by novel dialysis membranes, as well as the effect of new drugs for the inhibition of their formation.

β_2-Microglobulin isolated from the amyloid deposits in patients with dialysis-related amyloidosis has been demonstrated to be modified with AGEs,

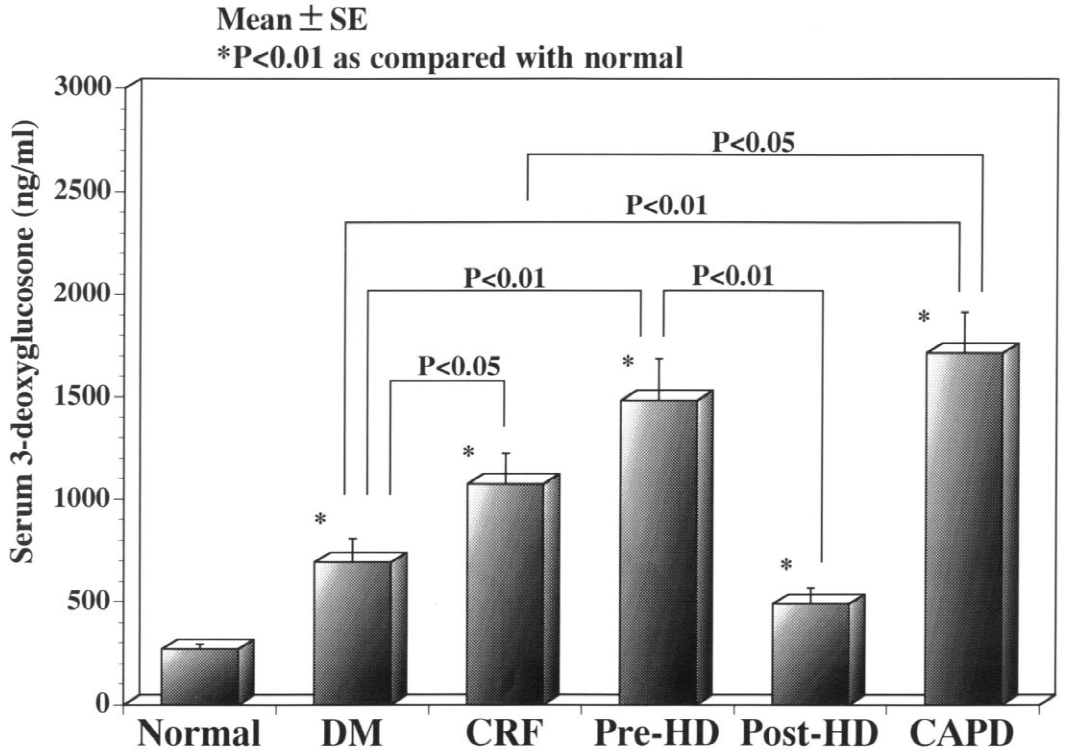

Fig. 5. Serum levels of 3-DG in normal subjects, diabetic patients (DM), undialyzed uremic patients (CRF), hemodialysis (HD), and continuous ambulatory peritoneal dialysis (CAPD) patients.

such as imidazolone and CML [23,32,70]. Further, we demonstrated that imidazolone and CML were localized to amyloid deposits in patients with dialysis-related amyloidosis by immunohistochemistry using monoclonal anti-imidazolone and anti-CML antibodies [21,32,70]. Incubation of β_2-microglobulin with 3-DG under physiological conditions emitted fluorescence characteristic for AGE (excitation, 370 nm; emission, 440 nm), and caused AGE modification and dimer formation of β_2-microglobulin as demonstrated by Western blotting using the same monoclonal antibodies. The AGE-modified dimer of β_2-microglobulin could be extracted from the amyloid tissue of a patient with dialysis-related amyloidosis. 3-DG showed more intense and faster reactivity with β_2-microglobulin to form AGEs (imidazolone and CML) and dimer compared with glucose, and aminoguanidine suppressed the AGE and dimer formation of β_2-microglobulin by 3-DG.

3-DG accumulating in uremic serum promotes the modification of β_2-microglobulin with AGEs including imidazolone and CML mainly after deposition of β_2-microglobulin as amyloid [69]. Once amyloid is formed, it is difficult to degrade. Then, it may react with 3-DG and the other precursors of AGEs, and become modified with imidazolone and CML even in normoglycemia after a long-term period of deposition as β_2-microglobulin amyloid. Further, a concomitant generation of oxygen radicals which occurs in hemodialysis patients, may enhance the aggregation of amyloid β_2-microglobulin [71,72]. Thus, a uremic state in which 3-DG is accumulated [22,56] and generation of oxygen radicals is increased, accelerates the AGE modification and aggregation of β_2-microglobulin amyloid.

6. Conclusions

3-DG was originally detected as a reactive intermediate of the Maillard reaction in vitro. However, recent studies have demonstrated that 3-DG is synthesized in human bodies via the Maillard reaction and the polyol pathway, and is involved not only in

aging but also in the development of diabetic and uremic complications. Several enzymes that detoxify 3-DG to 3-deoxyfructose or 2-keto-3-deoxygluconic acid were purified from animal organs. The plasma levels of 3-DG which have been quantified using GC–MS or HPLC, and their levels were found to be increased not only in uremic patients but also in diabetic patients. The research on AGEs as a pathogenetic factor for diabetic and uremic complications is at best progressing slowly, because effective inhibition of AGE formation has not yet been achieved in vivo. 3-DG rapidly reacts with protein amino groups to form AGEs, such as imidazolone, and furthermore it has some biological activities. The involvement of 3-DG in modification of tissue proteins has been demonstrated in diabetic and uremic patients by immunohistochemistry and immunochemistry using a monoclonal anti-imidazolone antibody. These recent studies have highlighted a crucial role of 3-DG in the development of diabetic and uremic complications.

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