理學碩士學位請求論文

Dictyostelium discoideum 의 생장에 미치는 포도당의 영향

Effect of glucose in the growth of *Dictyostelium discoideum*

2015 年 **2** 月

서울大學校 大學院

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Effect of glucose in the growth of

Dictyostelium discoideum

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ABSTRACT

The glycation of proteins by glucose has been linked to the development of diabetic complications and other diseases. Early glycation is thought to involve the reaction of glucose with N- terminal chain amino groups to form Schiff's base and fructosamine adducts. The formation of the α-oxoaldehydes, glyoxal, methylglyoxal, in early glycation was investigated. Glucose degraded slowly to form glyoxal, methylglyoxal. Cells were cultured with high concentration of glucose in HL5 media showed cell growth inhibition and increase of the cell size and weight. And concentration of intracellular methylglyoxal measured drastically increased during cultured with high-dose glucose in media. This results supporting the high-dose glucose induced accumulation of methylglyoxal *in vivo*. To confirm this hypothesis, putrescine added under the same incubate conditions, and the result exhibited putrescine blocks cell growth inhibition caused by accumulation of methylglyoxal. These results suggest a possibility of accumulation of methylglyoxal from high-dose glucose.

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LIST OF ABBREVATIONS

AGEs	advanced glycation end-product
CAD	cadaverine
HPLC	high performance liquid chromatography
MG	methylglyoxal
OE	overexpression
PUT	putrescine
SPD	spermidine
SPM	spermine
ODC	ornithine decarboxylase

I. INTRODUCTION

1. Glucose

1.1 An overview

Glucose is a simple aldosic monosaccharide found in plants. It is a monosaccharide that is absorbed directly into the bloodstream during digestion. It is an important carbohydrate in biology, which is indicated by the fact that cells use it as a secondary source of energy and a metabolic intermediate. Glucose is one of the main fuels for cellular respiration.

1.2 Glycation

Glycation, non-enzymatic glycosylation, is an endogenous process that contributes to the post-translational modification of proteins. It is slow under physiological conditions, giving rise to the presence of lysine- and arginine-derived glycation adducts in cellular and extracellular proteins. Inside cells, the impact of glycation is countered by high turnover and short half-life of many cellular proteins. Long-lived extracellular proteins, however, accumulate glycation adducts with age. Some of these adducts may be removed by enzymatic repair mechanisms, whilst all are removed by degradation of the glycated protein. Degradation of extracellular glycated proteins requires specific recognition by receptors, internalization and proteolytic processing. There are specific receptors, AGE receptors, which fulfil this role. Glycation of proteins has been linked to mechanisms of disease, particularly the development of chronic clinical complications associated with diabetes mellitus, retinopathy, neuropathy and

nephropathy, non-diabetic nephropathy, macrovascular disease, Alzheimer's disease, cataract and ageing.

2. Methylglyoxal

Methylglyoxal (MG), also called pyruvaldehyde or 2-oxopropanal, is the aldehyde form of pyruvic acid that has two carbonyl groups. MG is formed as a byproduct of several metabolic pathways (Inoue & Kimura, 1995) in various organisms. The most important source of MG is glycolysis. And it also may form from 3-amino acetone, which is an intermediated of threonine catabolism as well as through lipid peroxidation.

2.1. Toxicity by methylglyoxal *in vivo*

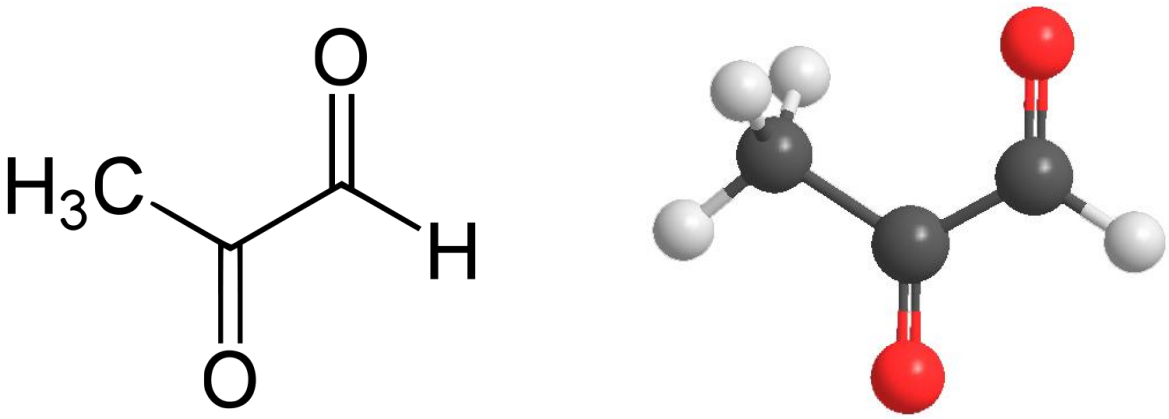
2.1.1. Advanced glycation end-products (AGEs)

A sugar aldehyde or ketone can react with an amino group of proteins, and also with phospholipid and nucleic acids giving rise to a Schiff base. This reaction occurs until reaching the equilibrium because it is reversible. The point of this reaction is the Schiff base is slowly rearranged giving the called Amadori product, which is fructoseamine. This is the early glycation process and the compounds formed are considered early glycation adduct. And they may to be undergo a further rearrangement and eventually dehydration, condensation, fragmentation, oxidation and even cyclization reactions. For this reason, the reaction is cannot be perfectly reversible, the so-called Advanced Glycation End-products (AGEs). In the pathogenesis of diabetes-related AGE formation, hyperglycemia results in higher

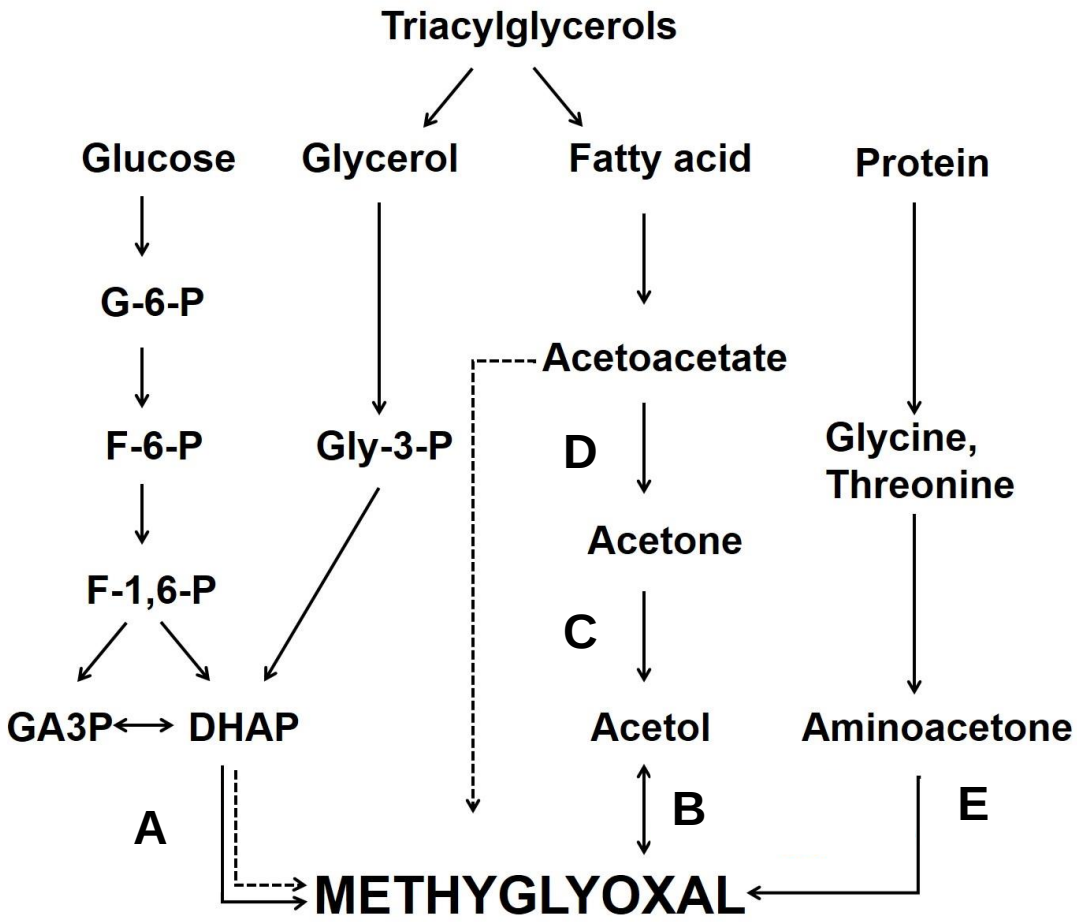
cellular glucose levels in those cells unable to reduce glucose uptake (Dominiczak, 2003; Brownlee, 2005). More AGEs are formed and production of AGEs when blood sugar is increased. Synthetically, AGEs are involved in not only physiologically aging but neurodegenerative diseases, such as Alzheimer disease (AD) and Parkinson disease (PD). We previously reported that PUT react with methylglyoxal to make PUT-MG adduct.

2.1.2. Inhibition of cell growth by methylglyoxal

Szent-Györgyi suggested that glyoxal derivatives acts as a growth-inhibiting factor, called retine, whereas glyoxalse promotes cell growth, called promine (Szent-Györgyi *et al.,* 1967). The reaction of MG with guanylate also formed N^2-(1-carboxyethyl) guanylate (Papoulis *et al.,* 1995). The modification of guanyl residues in DNA and RNA is thought to contribute to the anti-proliferative activity of MG (Együd and Szent-Györgyi, 1966; Apple and Greenberg; 1967, Jerzykowski *et al.,* 1970; Conroy *et al.,* 1979), which is characterized by inhibition of DNA synthesis (Reiffen and Schneider, 1984) and inhibition of translation initiation (White and Rees, 1982). DNA modification by MG induced single-strand breaking down, DNA to protein cross-links and even cytotoxicity (Marinari *et al.,* 1984). Some other alternative research reported MG inhibited tumor cell growth by inhibition of glycolysis and mitochondrial respiration, particularly by inhibition of tumor cell G3PDH (Ray *et al.,* 1991; Halder *et al.,* 1993). MG induced growth arrest in the G1 phase of the cell cycle and toxicity in human leukemia 60 cell *in vivo* and incubation of human leukemia 60 cell with MG led to the rapid accumulation of MG with DNA (Kang *et al.,* 1996).



Scheme 1. Chemical structure of methylglyoxal



Scheme 2. Production of methylglyoxal. G-6-P, Glucose-6-phosphate; F-6-P, Fructose-6-phosphate; F-1, 6-P, Fructose-1,6-biphosphate; Gly-3-P, Glycerol-3-phosphate; GA3P, Glyceraldehydes-3-phosphate; DHAP, Dihydroxyacetone phosphate. Enzyme: A. Methylglyoxal synthase; B. Acetol monooxygenase; C. acetone monooxygenase; D. Acetoacetate decarboxylase. E. Amine oxidase. Dotted line presents the pathway of non-enzymatic MG production (Kalapos, 1999).

3. Polyamines

3.1 An overview

The polycationic polyamines as 1, 3-diaminopropane, cadaverine, diamine putrescine, triamine spermidine and tetraamine spermine are small molecules found in all cells (Tabor and Tabor, 1984; Agostinelli, 2010). These molecules are reported to be involved in the control of various biological processes, including transcription, translation, enzyme activity, regulation of ion channel and the response to oxidative stress (Pignatti *et al.,* 2004; Wallace, 2009). It is capable to synthesize polyamines from a precursor amino acid, arginine and methionine in various living organisms (Davis *et al.,* 1992; Morgan, 1999). In previous study known that polyamines are essential for cell growth including their rate of synthesis and even total content increase proportionately with increase in cell proliferation (Porter and Bergeron, 1983). All those study meant to polyamine affects various cell processes, intracellular pools are ordinarily maintained within a relatively narrow range through control of anabolism and catabolism and import to export (Alhonen-Hongisto *et al.,* 1980). We give importance to putrescine to study in polyamines.

3.2. The enzymatic synthesis of polyamines

Most living organisms have polyamines in particular all polyamines have been found in eukaryotes, but SPM is less common in prokaryotes. Synthesis of polyamines in a large number of organisms is considerable similar, but there are differences of detail between the biosynthetic pathways depends on

different cell types, and a generalized pathway of biosynthesis of polyamine. In mammalian cells and in fungi the initial and at this stage rete-limit in step is the decarboxylation of ornithine to form PUT. It catalyzed by ornithine decarboxylase (ODC) which required for the first stage in polyamine synthesis. Synthesized PUT by ODC pathway can transform to SPD arise from addition of a group of aminopropyl donated by decarboxylated S-adenosylmethionine. This reaction catalyzed by the aminopropyltransferase, SPM synthase. Different aminopropyltransferase, SPD synthase, catalyze the addition of second group of aminoprooyl to SPM formation.

3.3. Ornithine decarboxylase

ODC catalyzed the incipient step in the biosynthesis of polyamine, small, ubiquitous, abundant, and essential cellular polycation. ODC has an unconditional requirement for pyridoxal 5'- phosphate for activity. The binding site for pyridoxal phosphate is at lysine-69 in mouse ODC (Murakami *et al.,* 1989), which is in a highly conserved region within the eukaryotic enzymes. ODC protein has a half-life of the order of 10-60 minutes the differences of detail appear depending on species, but in any case, as we known it has an exceedingly short existence period. The carboxyl terminal part of mouse ODC contains a PEST region (Rogers *et al.,* 1986). The only function of ODC appears to be to catalyze the decarboxylation of ornithine to form PUT, the initial diamine in the polyamine biosynthetic pathway. Activity of ODC is extremely low and it has been evaluated that there may be sole 100-200 molecules of enzyme in a quiescent cell in normal cell conditions (Pegg *et al.,* 1982). ODC can perform to both positive/negative feedback which regulated by polyamines. High concentration of polyamines decrease activity and obviously low concentration of polyamines make a increase of the activity. The ODC

promoter contains transcription factor response elements that are activated upon stimulation with hormones or growth factor. As we saw, polyamines are required for cell growth (Janne and aina, 1969; Bello-Fernandez *et al.,* 1993).

3.4. Polyamines in *Dictyostelium discoideum*

In *Dictyostelium*, the major polyamines found in vegetative cells are PUT (5-10 mM), SPD (2 mM) and 1 ,3-diaminopropane (5 mM), but no SPM was found at growth phase encompass developmental stage (Mach *et al.,* 1982, Klein *et al.,* 1990). Alike ratio of polyamines, High rate of ODC activity during vegetative cells. It have a thread of connection with ODC regulates a concentration of polyamines. Upon starvation, the ODC activity expeditiously decrease (Turner *et al.,* 1979) and there are no significant in ODC activity was found by these authors during further differentiation. On the other hands, PUT and 1, 3-diaminopropane appears

3.5. Putrescine

Putrescine (PUT), also called tetramethylenediamine, is a foul-smelling organic chemical compound $NH_2(CH_2)_4NH_2$ (1 ,4-diaminobutane or butanediamine) that is related to cadaverine. Their most important pathway of production is the breakdown of amino acids in living and dead organisms. Large doses of polyamines cause toxic effects in all cases. The two compounds are largely responsible for the foul odor of putrefying flesh, at the same time contribute to the odor of such processes as bad breath and bacterial vaginosis. They are also exist in some microalgae and semen, together with related molecules like spermine and spermidine.

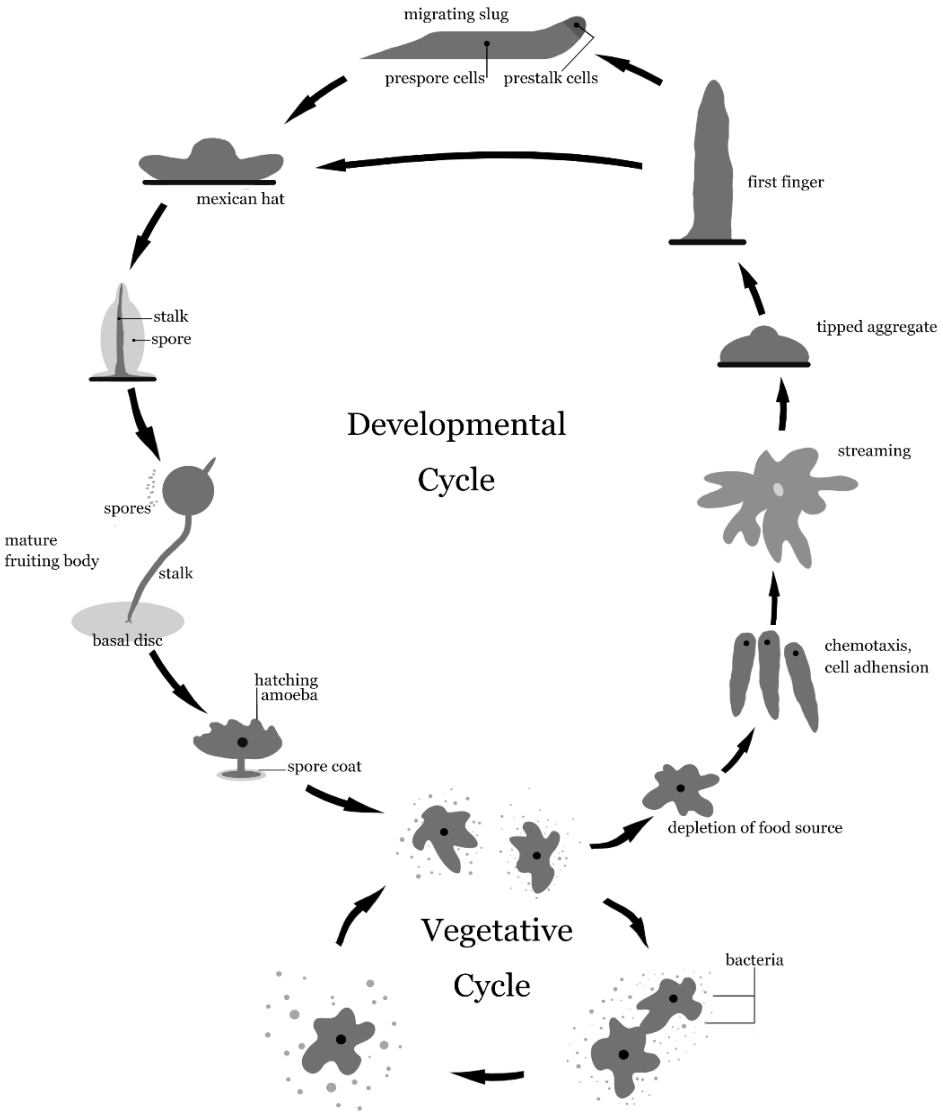
4. *Dictyostelium discoideum*

4.1. An overview

Dictyostelium discoideum is the most studied species of the social amoeba, which are also known as the cellular slime molds. It is a soil-living amoeba that grows as a unicellular organism, feeding on bacteria and multiplying by binary cycle to form a fruiting body consisting of obviously two distinct cell types that stalk cells and spores. The initial response to starvation is for the cells to be collected in multicellular aggregates by chemotaxis in response to periodically generated pulses of cAMP. Each aggregates then elongates to form a migrating pseudoplasmodium or slug, which is sensitive to heat, light and humidity. Initial differentiation of randomly dispersed cells into prestalk and prespore cells is hardly distinguishable in the aggregate. However, the cells sort out chemotactically during tip formation and by the slug stage the prestalk cells occupy the anterior 20% with the prespore cells ay the back of the slug (Loomis, 1993). Spores germinate under favorable conditions and single amoebae emerge, whereas stalk cells die during the differentiation process.

4.2. Cell cycle during vegetative growth

Dictyostelium discoideum cells are haploid and contain six chromosomes. They can grow on bacteria (e.g. *Klebsiella aerogene* or *Escherichia coli*), either in suspension or on agar plates, with generation time of 3-4 hr. Axenic mutants have been isolated. That can be grown in either



Scheme 3. ***Dictyostelium discoideum*** **life cycle.** Starvation triggers individual amoeba to aggregate so that after 15 h, amoeba aggregate into mounds of cell. Then a tip appears at the apex of mound and elongated vertically to form a finger-like structure.

a complex nutrient medium, where they double every 10 hours, or in a defined medium where they double every 10-24 hr., depending on the strain (Franke and Kessin, 1977). The cell cycle of growing axenic cells has been analyzed by classical techniques and by measurement of the DNA content of cells and nuclei by flow fluorometric techniques.

5. Aims of this study

Szent-Györgyi suggested that glyoxal derivatives acts as a growth-inhibiting factor, called retine, whereas glyoxalase promotes cell growth, called promine (Szent-Györgyi *et al.,* 1967). Toxic effects of MG are already well known by many publications. This study aim to how to regulate toxicity by polyamines, especially PUT. We previously reported by EPR (Electron paramagnetic resonance) that MG formed PUT-MG adduct with PUT. It suggests intracellular abnormally high concentrated MG will reduced by reaction with PUT. This study provides evidence that intracellular MG which was regulated by PUT is an endogenous growth inhibition factor in *D. discoideum*.

II. Material & Methods

1. Materials

G418 and blastcidin S were from Duchefa. Ampicillin, perchloric acid, putrescine, sodium acetate, methanol, dethylglyoxal, 2-methylquinoxaline and Propidium iodide were purchased from Sigma-aldrich. All other chemicals used were of the highest quality generally available.

2. Strains and culture conditions

2.1. *Dictyostelium discoideum* strains

The axenic *Dictyostelium discoideum* strain wild-type KAx3, ODC overexpression (*odc*^{oe}: Act15-*odc*^{oe}) in wild-type KAx3 and *odc* disruption (*odc*$^{-}$) cells were used to examine the function of PUT during vegetative growth (Choi *et al.,*2008).

2.2. Culture conditions in liquid medium

Dictyostelium discoideum wild-type KAx3, *odc*^{oe}, *odc*$^{-}$ cells were grown in HL5 (FOREDIUM) (Cocucci and Sussman, 1970; Franke and Kessin, 1977), which was supplemented with 200 μg/ml of streptomycin sulfate and 200 unit/ml of penicillin to prevent contamination, shaken 150 rpm at 22°C. For liquid the culture, *odc*$^{-}$ cells were maintained in HL5 media supplemented with 1 mM PUT. Depletion of intracellular PUT was accomplished by washing more than twice with fresh HL5 media. For culture for *odc*^{oe} cells, HL5 medium supplemented with G418 (20 μg/ml) was used on six well

Table 1. *D. discoideum* strains used in this study

	Genotypes or description	References of sources
	***Dictyostelium discoideum* strains**	
KAx3	Axenic wild-type strain	Firtel, 1997
odc^{-}	KAx3 : [ODC-Bsr], bs^{r} odc-disrupted KAx3	Park, 2008
odc^{oe}	KAx3 : [EXP (+)-ODC], neo^{r} ODC-overexpression KAx3	Park, 2008

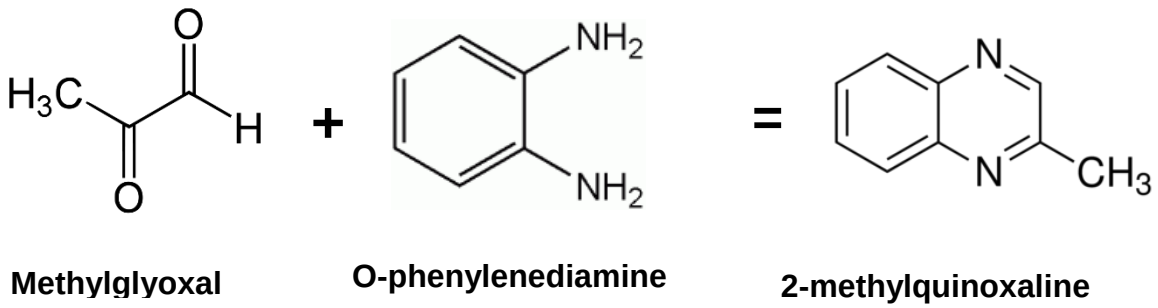
culture plates. And for *odc*$^{-}$ cells, HL5 medium supplemented with blastcidin S (10 μg/ml) and 1 mM PUT were used in six well culture plates. For strain maintenance, stock cells were prepared in 10% DMSO-HL5 and stored at lower than -70°C. For normally renewal of cell strain, frozen stock cells were thawed and suspended in 10 ml HL5 culture dish plate.

2.3. Culture conditions in liquid medium with high concentration of glucose

Dictyostelium discoideum wild-type KAx3, *odc*^{oe}, *odc*$^{-}$ cells were grown in HL5 with 74.93 mM, 149.86 mM, 224.79 mM, 299.72 mM, and 374.65 mM the concentration of glucose to induce methylglyoxal.

3. Measurement of intracellular methylglyoxal

Intracellular MG level was determined according to the methods (Chaplen *et al.,* 1996) with modification of some details. Exponentially growing cells were harvested and suspended in 0.5 M perchloric acid (PCA) to breaking the cell membrane and prevent to other unexpected further reactions with acidified cell substances. After incubation for at least 20 min in ice, the pellet was removed by centrifugation and the supernatant was incubated with 1 mM o-phenylendiamine at 65°C for 3 hours in the dark. The sample was passed through C-18 Solid phase (SPE : SEPPAK) extraction column which was activated with 5 ml methanol, washed with 5 ml purified water. And then it was eluted with methanol. The same was evaporated and dissolved in the mobile phase buffer which is 65% methanol,



Scheme 4. Methylglyoxal derivatives. 2-methylquinoxaline

30% purified water and 5% acetonitrile. The sample was separated on ZORBAX SB-C18 column using Agilent 1200 series high performance liquid chromatography (HPLC) system. Quinoxaline derivatives were detected at 336 nm. The amount of 2-methylquinoxalne, a quinoxaline derivative of MG was quantified by integration relative to the internal standard.

III. Results

1. Growth inhibition by high-dose glucose

Glucose is common compound to cell growth but precious report suggested abnormally high concentration of glucose arouse the growth inhibition (Choi *et al., 2008*). Therefore I had to find the point of toxicity glucose concentration. Wild-type KAx3 cells are well grown in HL5 complex media. 74.93 mM glucose condition is most compatible to growing but the study required addition of glucose, therefore cells were cultured in each five different concentration of glucose into the state. Condition on over the 224.79 mM, growth of cells were inhibited that is the reason what I decided the point of compatible glucose concentration (Fig. 1). The result suggested high-dose of glucose induced cell growth inhibition.

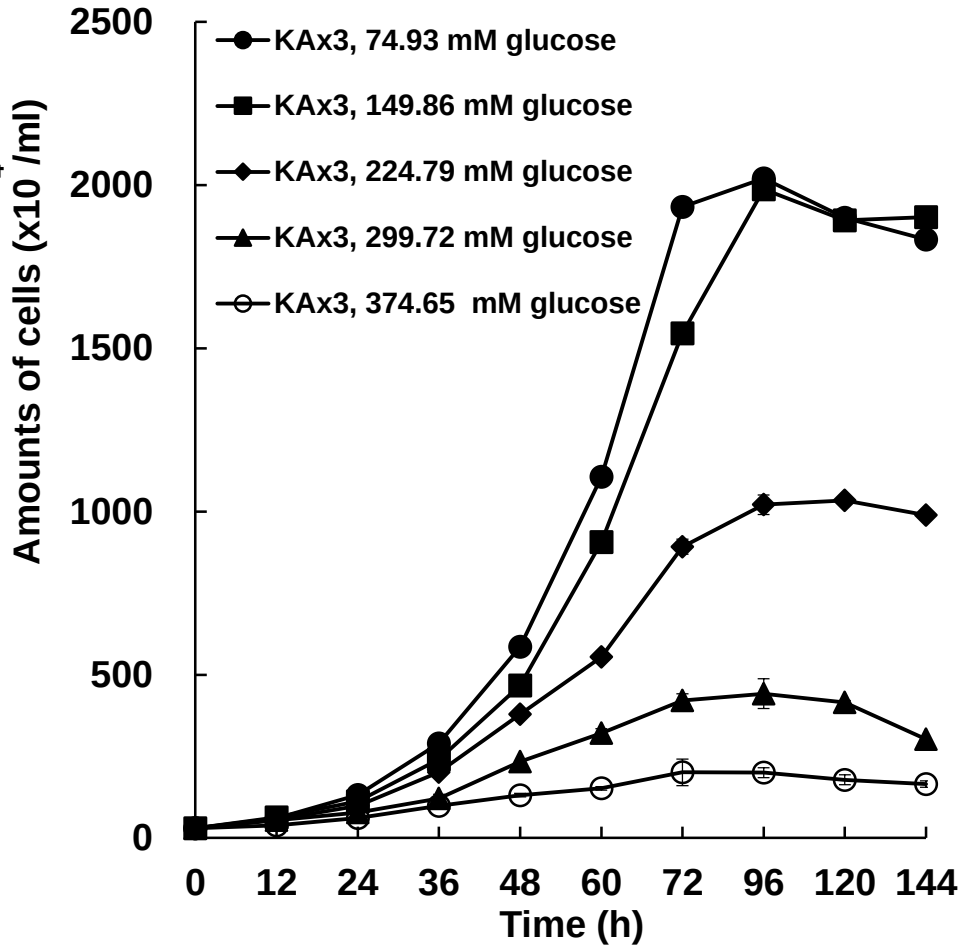


Fig. 1. Growth inhibition by high-dose glucose. The wild-type KAx3 cells cultured in the presence of five different concentrations (74.93 mM, 149.86 mM, 224.79 mM, 299.72 mM, and 374.65 mM) of glucose in HL5 media. Each points represent the average (± standard deviation) of three independent experiments.

2. Methylglyoxal accumulation by glucose in *D. discoideum*

MG has been known to be an endogenous metabolite inhibiting cell growth (Szent-Györgyi *et al.,* 1967, Kang *et al.,* 1996). To find out glucose induced accumulation of MG *in vivo*, wild-type KAx3, *odc*^{oe} and *odc*$^{-}$ cells were cultured in the 74.93 mM, 149.86 mM, 224.79 mM and 299.72 mM glucose added media. Each cell was harvested 1 day after from inoculated. Wild-type KAx3 cells that cultured in 224.79 mM glucose showed increasing about 50% more than with 74.93 mM glucose. Interestingly, with over 224.79 mM glucose, MG was decreased about 22% compared to lower than that the cells were cultured in the presence of 224.79 mM glucose in the medium. Because over 224.79 mM glucose, cell growth were extremly inhibited that means cell death. Levels of MG in *odc*^{oe} cells that cultured 74.93 mM glucose showed less concentration of MG compared with wild-type KAx3, and in 224.79 mM glucose, levels of MG were lower than wild-type KAx3. This results suggested possibility of the *odc*^{oe} cells were block the methylglyoxal accumulation by PUT. In *odc*$^{-}$ cells showed similar levels of MG to wild-type KAx3 cells and the condition of that cultured in 224.79 mM glucose exhibited highest concentration (Fig. 2).

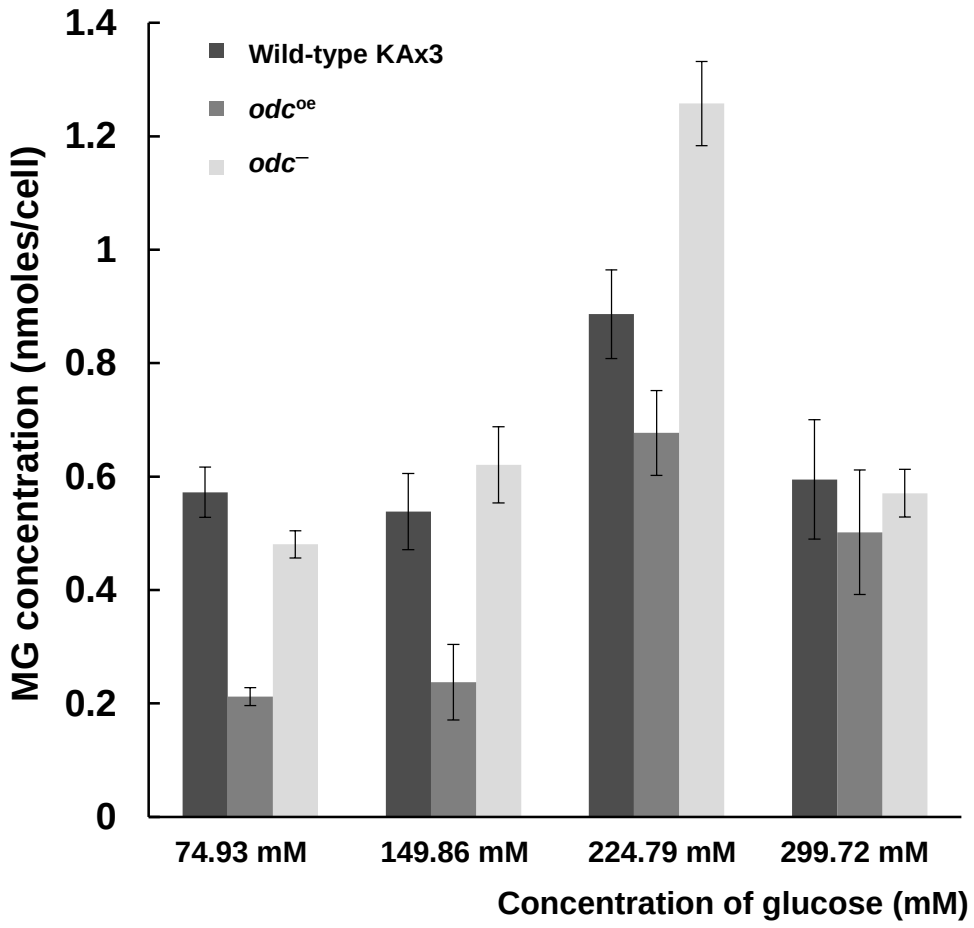


Fig. 2. Comparison of methylglyoxal accumulation among wild-type KAx3, *odc*⁻ and *odc*^{oe} cells. Cells were grown in the presence of 74.93 mM, 149.86 mM, 224.79 mM and 299.72 mM glucose and incubated in HL5 media. After 1 day culture, cells were harvested and MG levels were measured. The values represent the average (±standard deviation) of three independent experiments.

3. Cell weight variation from cell cycle arrest caused by methylglyoxal accumulation

Accumulation of MG is a primary factor inducing cell cycle arrest in G1 phase in *Dictyostelium*. Cell size expansion was already checked and it suggests cell weight increasing by MG accumulation *in vivo*. To confirm this hypothesis, wild-type KAx3 cells cultured with 74.93 mM, 149.86 mM and 224.79 mM glucose in media. Cells that cultured with high concentrated glucose in media were slightly increased. Interestingly, the cells that cultured in the presence of 224.79 mM glucose in medium increase drastically relatively other conditions (Fig. 5). With cell size expansion, this result supporting the cell cycle arrest by methylglyoxal accumulation caused by high-dose glucose.

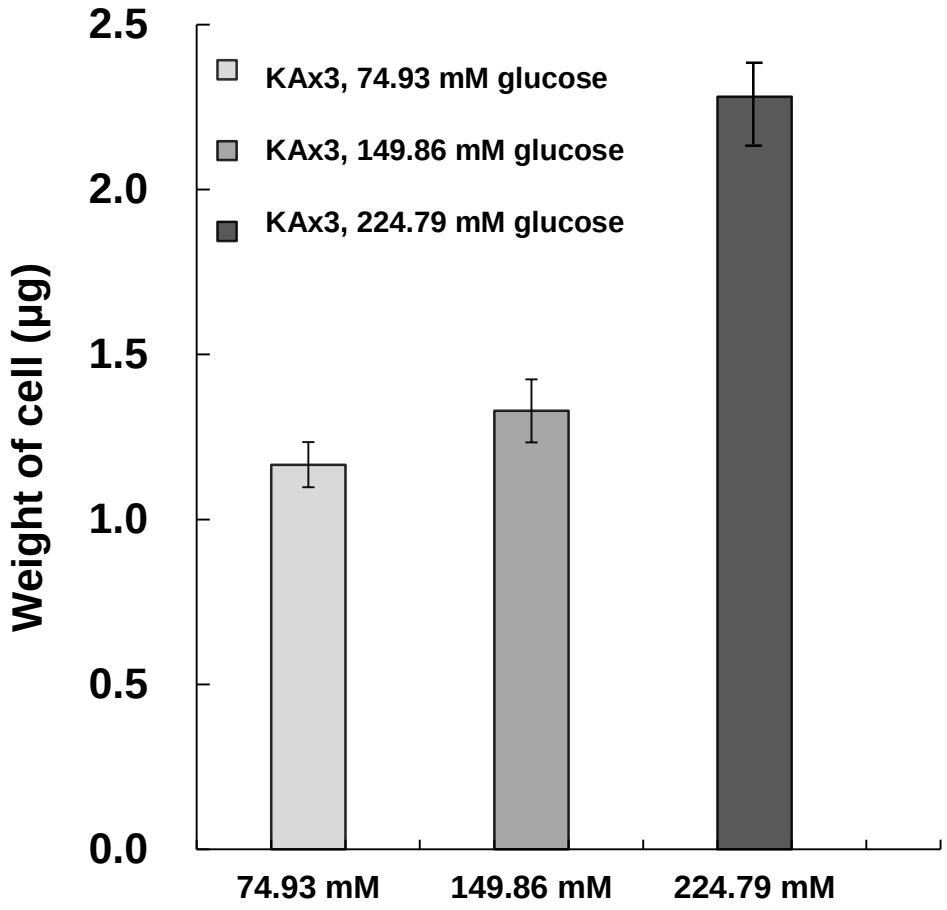


Fig. 3. Cell weight variation from cell cycle arrest caused by methylglyoxal accumulation.

Wild-type KAx3 cells were incubated at 3 x 10^5 cells/ml in the media with 74.93 mM, 149.86 mM and 224.79 mM glucose were measured using a microbalance at the exponential stage. Each cell represents the average (± standard deviation) of three independent experiments.

4. Cell size expansion from cell cycle arrest caused by methylglyoxal accumulation

In our previous study, MG accumulation induced as a primary factor for G1 cell cycle arrest in *Dictyostelium* (Choi *et al., 2008*). Cell size expansion is arisen by cell cycle arrest in G1 phase, because of failure of the cell division. To confirm this hypothesis, wild-type KAx3 cells cultured with 74.93 mM or 224.79 mM glucose in media. Cells that cultured with high concentrated glucose in media were slightly bigger, about 0.3 μm, than cells cultured in normal media (Fig. 3). Size of cells in the media of (Fig. 4 A) 74.93 mM and (Fig. 4 B) 224.79 mM glucose. The result suggested cell cycle arrest by methyglyoxal accumulation caused by high-dosed glucose.

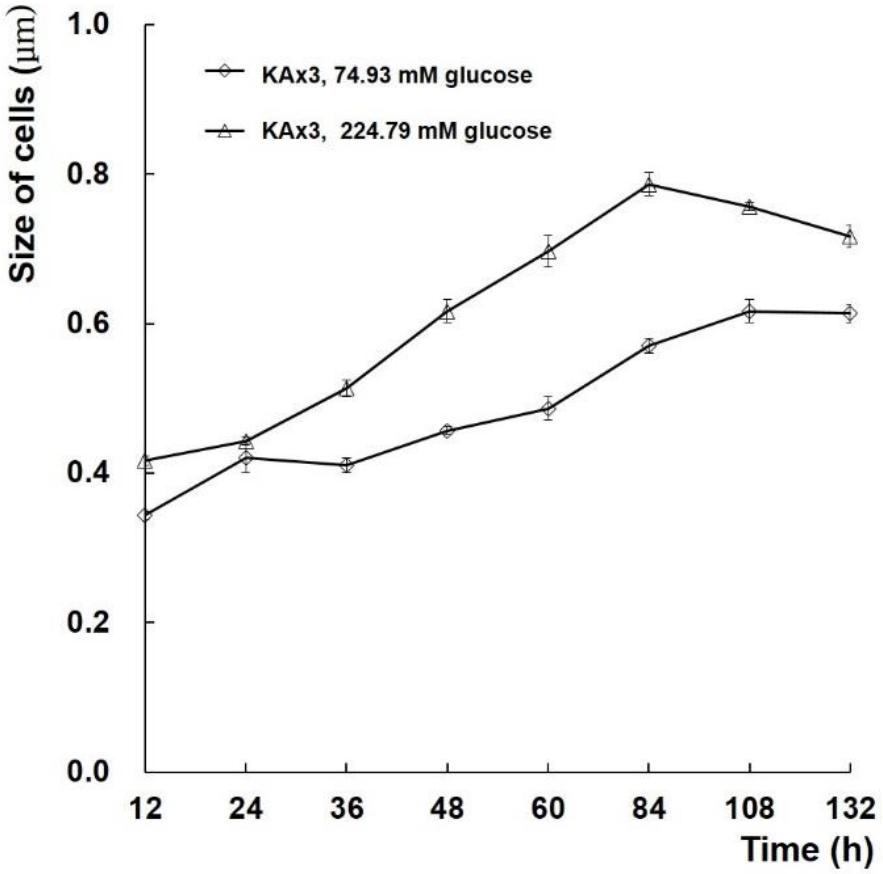
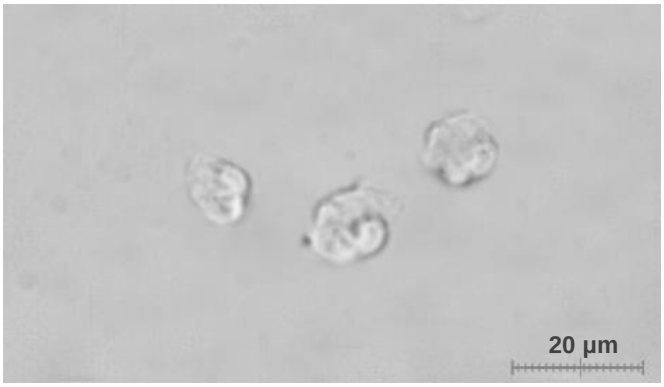


Fig. 4. Variation of cell size in KAx3 by glucose. Wild-type KAx3 cells were incubated at 3 x 10^5 cells/ml in the media with 74.93 mM glucose (open rhombus), 224.79 mM glucose (open triangles) was measured using an optical microscope with scale bar at the indicated times (h). Each point represents the average (± standard deviation) of three independent experiments.

A



B

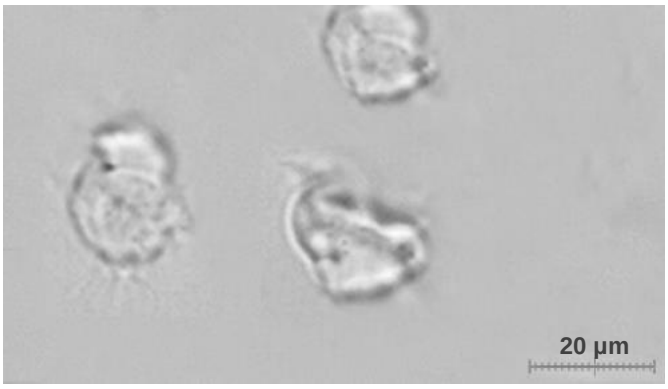


Fig. 5. Variation of cell size in KAx3 by glucose. Wild-type KAx3 cells were incubated in the media with 74.93 mM glucose, 224.79 mM glucose was measured using an optical microscope.

5. Growth regulation by putrescine in KAx3, *odc*^{oe} and *odc*$^{-}$ cells in the additional glucose media

To find out the role of PUT in cell growth inhibited by methylglyoxal, 0.1 mM PUT were treated in media. Cells were incubated in the media with 74.93 mM or 224.79 mM glucose, and 0 mM or 0.1 mM PUT were treated. There were no significant difference between presence of 0 mM and 0.1 mM PUT that wild-type KAx3 and *odc*^{oe} cells cultured in HL5 media (Fig.6 A, B). PUT is an essential compound for cell growth in *Dictyostelium*. Hence growth of *odc*$^{-}$ cells cultured without PUT were inhibited. However, the presence of 0.1 mM PUT grew to normal case (Fig. 6 C). To induct methylglyoxal, additional glucose was added in HL5 media. *odc*^{oe} cells showed partial recovery larger than wild-type KAx3 cells (Fig. 6 A, B). Understandably *odc*$^{-}$ cells cultured without PUT dose not showed growth, but in the presence of 0.1 mM PUT recovered drastically as compared with 0 mM PUT (Fig. 7 C). The result suggested PUT blocks growth inhibition from toxic effects of MG. To find out PUT blocks accumulation of MG *in vivo*, wild-type KAx3, *odc*^{oe} and *odc*$^{-}$ cells were cultured in the glucose added media with 0 mM or 0.1 mM PUT. Each cell was harvested 1 day after from inoculated. Wild-type KAx3 cells that cultured in 224.79 mM glucose without PUT showed increasing about 50% more than with 74.93 mM glucose. Interestingly, with 0.1 mM PUT, MG was decreased about 22% compared to without PUT in the presence of 224.79 mM glucose in the medium. Levels of MG in *odc*^{oe} cells that cultured 74.93 mM glucose without PUT showed

less concentration of MG compared with wild-type KAx3, and in 224.79 mM glucose without PUT, levels of MG was similar to wild-type KAx3 that cultured in the presence of 0.1 mM PUT. This results suggested possibility of the *odc*^{oe} cells produce about 0.1 mM PUT. In *odc*$^{-}$ cells showed similar levels of MG to wild-type KAx3 cells and the condition of that cultured in 224.79 mM glucose exhibited highest concentration of MG which was at least 1.2 nmoles/cell. However, *odc*$^{-}$ cells with 0.1 mM PUT in 224.79 mM glucose, levels of MG was drastically decreased compared with without PUT (Fig.5). All those results supported possibility of regulation of MG accumulation by PUT.

Fig. 6. Growth of wild-type KAx3, *odc*^{oe} and *odc*$^{-}$ cells with putrescine. 0 mM PUT (circles), 0.1 mM (squares). (A) Growth curves. Wild-type KAx3 cells were cultured with 0 mM, 0.1 mM PUT in HL5 media. (B) Growth curves. *odc*^{oe} cells ere cultured with 0 mM, 0.1 mM PUT in HL5 media. (C) Growth curves. *odc*$^{-}$ were cultured with 0 mM, 0.1 mM PUT in HL5 media. Each cells were counted using a hemacytometer at the indicated times (h). Each point represents the average (± standard deviation) of three independent experiments.

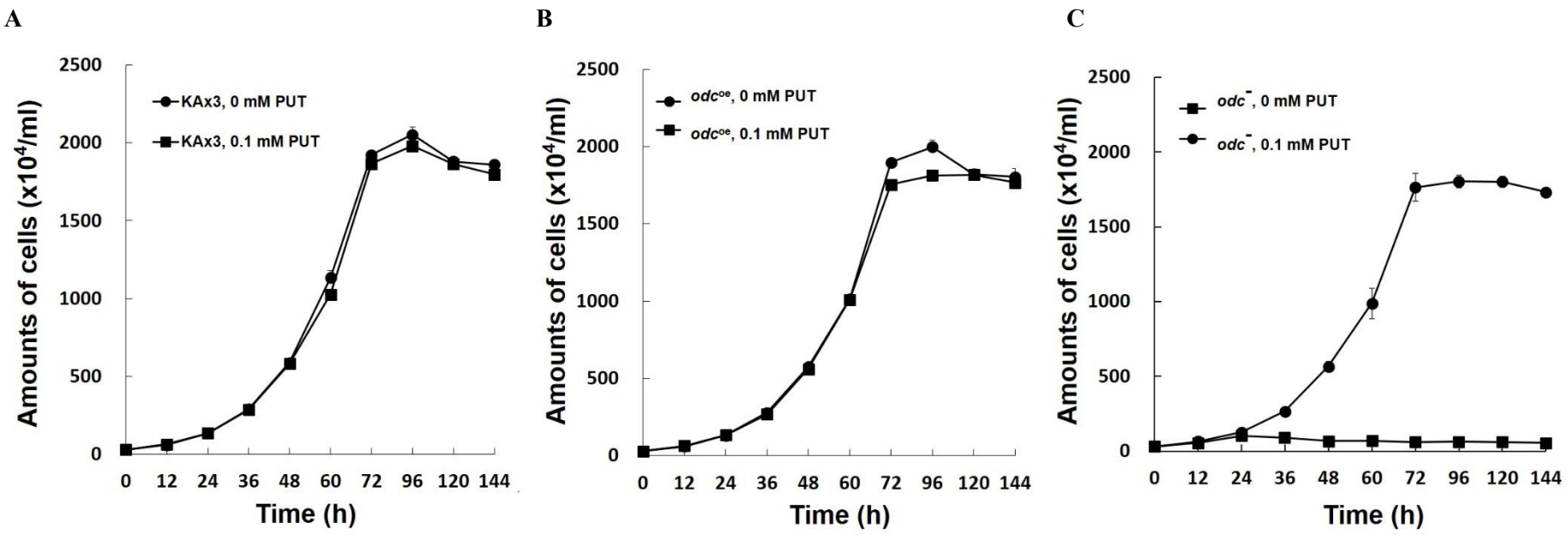
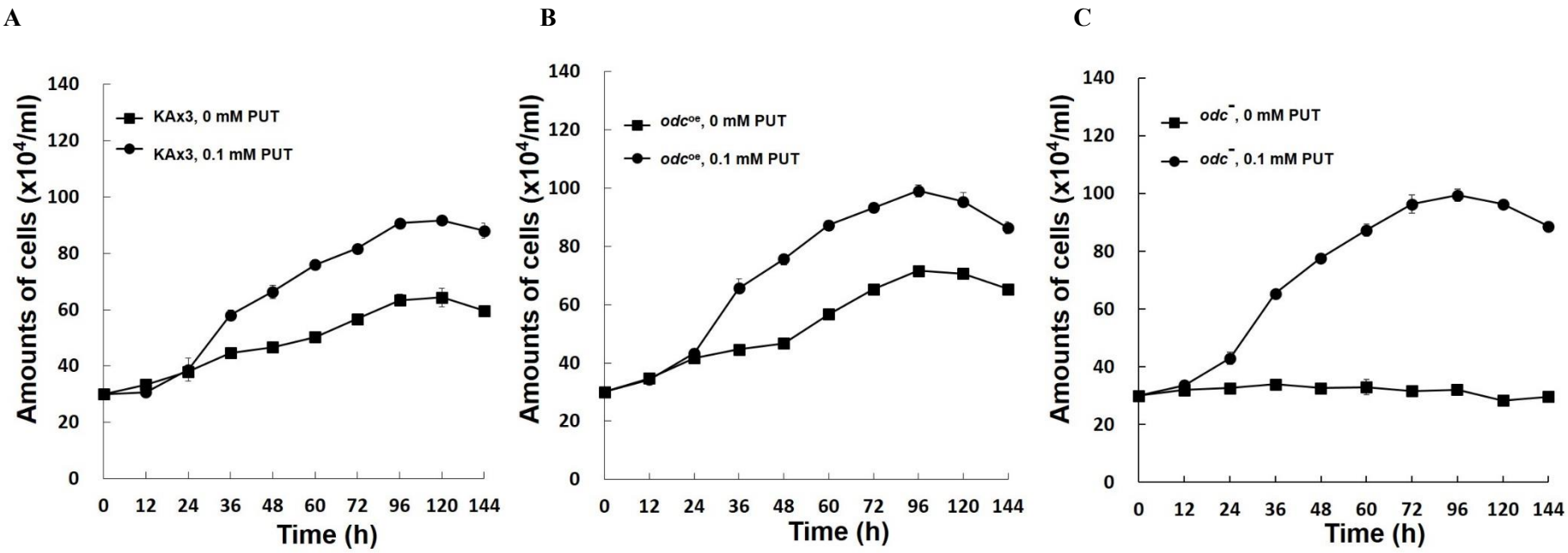

A
B
C
Amounts of cells (x10^4/ml)
Time (h)
KAx3, 0 mM PUT
KAx3, 0.1 mM PUT
odc^oe, 0 mM PUT
odc^oe, 0.1 mM PUT
odc^-, 0 mM PUT
odc^-, 0.1 mM PUT
0
500
1000
1500
2000
2500
0 12 24 36 48 60 72 96 120 144

Fig. 7. Growth of KAx3, *odc*^{oe} and *odc*$^{-}$ cells with putrescine. 0 mM PUT (circles), 0.1 mM (squares). (A) Growth curves. Wild-type KAx3 cells were cultured with 0 mM, 0.1 mM PUT in HL5 media with 224.79 mM glucose. (B) Growth curves. *odc*^{oe} cells ere cultured with 0 mM, 0.1 mM PUT in HL5 media with 224.79 mM glucose. (C) Growth curves. *odc*$^{-}$ were cultured with 0 mM, 0.1 mM PUT in HL5 media with 224.79 mM glucose. Each cells were counted using a hemacytometer at the indicated times (h). Each point represents the average (± standard deviation) of three independent experiments.


A
B
C
Amounts of cells (x10⁴/ml)
Time (h)
KAx3, 0 mM PUT
KAx3, 0.1 mM PUT
odc^oe, 0 mM PUT
odc^oe, 0.1 mM PUT
odc⁻, 0 mM PUT
odc⁻, 0.1 mM PUT
0 12 24 36 48 60 72 96 120 144
0 20 40 60 80 100 120 140

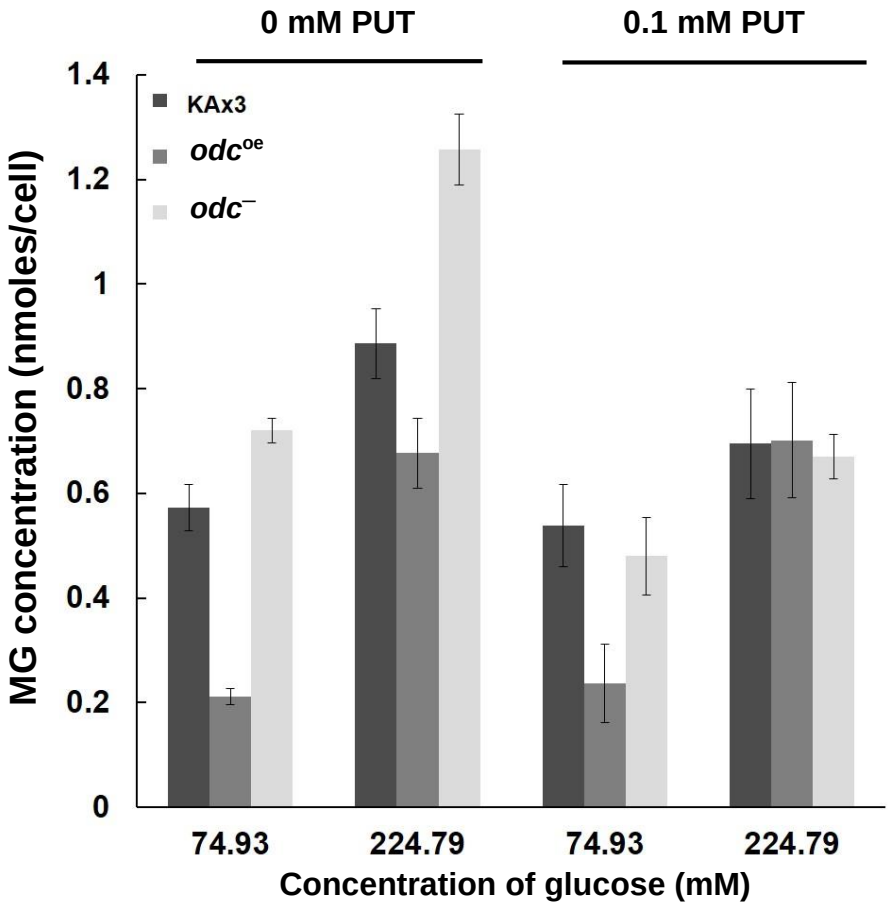


Fig. 8. Comparison of methylglyoxal accumulation among wild-type KAx3, *odc*⁻ and *odc*^{oe} cells with putrescine. Cells were grown in the presence of 74.93 mM or 224.79 mM glucose and incubated in the media with 0 mM or 0.1 mM PUT. After 1 day culture, cells were harvested and MG levels were measured. The values represent the average (±standard deviation) of three independent experiments.

IV. Discussion

MG is a toxic endogenous metabolite produced by non-enzymatic elimination of phosphate form glycolytic pathway intermediations. MG modifies DNA and proteins, thereby inhibiting cell proliferation (Kang *et* al., 1996) or inducing apoptosis (ukunaga *et al*., 2005). MG is a major glycation agent to form advanced glycation end products (AGEs), which has been implicated in diabetes complications (Fukunaga *et al*., 2005; Yim *et al*., 2001). As mentioned, MG produced by various pathways, however, most important pathway is a glycolysis. This study showed incubated cells with high-dose glucose induced cell growth inhibition and that produced high concentration of intracellular MG. This is the part that is directly related to the induction illuminate the effect of the MG via the high-dose glucose. Look forward to the future reveal the exact mechanism of generation of MG takes place in the glycation process. This study expected to be a great contribution to eliminating the toxicity methodology applied to the cells due to the MG with the previous reported thesis that addition of putrescine blocks methylglyoxal accumulation,

V. REFERENCES

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국문초록

글루코오스의 당화 단백질은 당뇨병 합병증 및 기타 질병의 발달과 관련이 있다. 당화는 Schiff-bass 와 fructosamine 이 물을 형성하기 위하여 N- 말단 아미노기와 글루코오스의 반응을 수반하는 것으로 생각된다. 당화에서 α-oxoaldehydes, 글리옥살, 메틸글리옥살은 글루코오스의 분해에 의해 형성된다는 보고가 있다. 고농도 포도당은 세포 내에서 메틸글리옥살 축적을 유도하여 세포 성장 억제를 유발한다. *Dictyostelium discoideum* 에서 고농도의 글루코오스는 세포의 크기와 질량을 증가시켰고 메틸글리옥살 축적과 이에 따른 세포 성장 억제를 유발하였다. 또한 putrescine 에 의한 회복효과를 확인하였다. 이것은 고농도 포도당이 세포 내 메틸글리옥살의 농도를 높여 독성 효과를 나타낼 수 있는 가능성을 제시하여준다.