22nd JMARS annual meeting
ABSTRACTS

December 21st (sat) , 22nd (sat)

Tokyo University of Agriculture and Technology
Green Hall,　Koganei campus

JMARS

Plenary lecture

Biomolecular Engineering of Enzymes for Diagnostics

Koji SODE
Graduate School of Engineering
Department of Bitoechnology
Tokyo University of Agriculture & Technology

Variety of oxidoreductases have been utilized as in the clinical diagnositcs. The most representative enzyme is glucose oxidase, which has been used for the blood glucose level monitoring, especially as the enzyme for the Self Monitoring of Blood Glucose (SMBG). These enzymes were used in electrochemical biosensors based on artificial electron acceptors. However, the measurements by electron mediator-type electrochemical monitoring systems employing oxidases are inherently influenced by the amount of oxygen dissolved in the sample. The high O_2 reactivity of oxidases therefore limits their potential utility for biosensor applications employing artificial electron mediators. In this presentation, our recent challenges in the biomolecular engineering of oxidases are presented. We developed essential "dehydrogenase" from oxidases, which are less O_2-sensitive, and with increased dye-mediated dehydrogenase activity.

Perspective of Melanoidin as Ultimate Maillard Reaction Products

Toshiharu, Gomyo
Kagawa Nutrition University (Joshi Eiyo Daigaku)

The Maillard reaction gives rise to a number of complex reactions via dehydration, oxidation, cyclization and scission during heating, and this results in discoloration (browning) of the reaction mixture, which not only occurs in processed and stored foods, but also plays a role in the formation of humic substances in the soil.

The overview of the Maillard reaction appears to be a “labyrinth” under construction, where the pathways are extraordinarily entangled with unknown intermediates, particularly in advanced stages. The ultimate stage of the Maillard reaction gives rise to higher polymeric and brown-colored pigments called melanoidins via interaction among reaction intermediates, such as dicarbonyls and amino compounds. Despite 100 years of work since Maillard’s initial description, vast unknown areas remain.

Keynote lectureII

Things I Learned From Statin Drugs Discovery

Akira Endo
Tokyo University of Agriculture and Technology

Biography

Dr. Endo discovered mevastatin, the first statin, pioneering research into a new class of molecules that are now a hugely successful class of drugs targeting the lowering of cholesterol. His work was done at Sankyo Company in Japan, and he is currently Director of Biopharm Research Laboratories and Distinguished Professor Emeritus at the Tokyo University of Agriculture and Technology.

(http://www.uspto.gov/news/pr/2012/12-15.jsp)

Recent winning awards:

Warren Alpert Foundation Prize (Harvard Medical School, U.S.A), 2000

Albert Lasker Award for Clinical Medical Research, 2008

Persons of Cultural Merit (Japan) 2011

The National Inventors Hall of Fame Inductee (U.S.A.) 2012

The Order of the Sacred Treasure, Gold and Silver Star (Japan) 2012

The National Academy of Inventors Charter Fellows (U.S.A), 2012

S-1

Screening of novel molecular recognition molecules for glycated protein measurement

○**Akane Sakaguchi-Mikami**

School of Biotechnology and Bioscience, Tokyo University of Technology

Glycated hemoglobin, Hemoglobin A1c (HbA1c) is a major indicator for diabetes mellitus. HbA1c is produced by a non-enzymatic reaction between glucose and N-terminal valine residue in the β-chain of hemoglobin. Fructosyl-valine (FV), one of a-fructosyl-amino acid (α-FA), is thus the target molecule released from HbA1c protease digestion.

In this paper, we report our attempt to investigate novel α-FA specific molecules by focused on an α-FA catabolism system from a gram-negative plant-pathogenic bacterium, *Agrobacterium tumefacience*. Our approach in the construction of novel biosensing methods for HbA1c based on these molecules will be also presented.

S-2

Evolution of glycated protein measurement methods in a clinical practice

Keiko Yasukawa

Diagnostics Department of ASAHI KASEI PHARMA CORPORATION

In 1960s, when electrophoresis of blood of a deabetic was carried out, it was discovered that there was a component which moved to a cathode side compared to usual hemoglobin. The development of the glycohemoglobin measurement method began from this discovery. The HPLC method was developed from the electrophoresis, and now various HbA1c measurement methods, such as immunological and enzymatic methods have been developed. The glycohemoglobin measurement methods have been standardized to HbA1c measurement as many methods have been developed. It is now defined that HbA1c is the hemoglobin by which beta chain N-terminal valine is glycated. In 1970s, Fructsamine measurement method was developed as a glycemic index which reflects a shorter-term blood sugar change than HbA1c, and in 1980s glycated albumin measurement method was developed to measure glycated albumin specifically. In the routine clinical examination, the measurement of glycated protein plays the leading role of the diabetes testing. As the importance of the measurement of glycated protein increases, not only accuracy, but also globally standardized measurement method are required

S-3

Selection of glycemic control indicator in accordance with the clinical conditions

Masafumi Koga

Department of Internal Medicine, Kawanishi City Hospital

Since plasma glucose fluctuate every day ever under the influence of meal, it is not possible to use it as a glycemic control indicator. Because glycated proteins are correlated with the past mean plasma glucose, they could be used as glycemic control indicators. Of these, glycated hemoglobin (HbA1c) and glycated albumin (GA) are used clinically. However, HbA1c and GA levels are also influenced by hemoglobin metabolism and albumin metabolism, respectively. Since half-life of albumin is shorter than hemoglobin (erythrocytes), GA reflects shorter-term plasma glucose (2-4 weeks) whereas HbA1c reflects plasma glucose levels for the past 1-2 months. HbA1c shows abnormal levels in patients with variant hemoglobin or hemolytic anemia. On the other hand, GA shows abnormal levels in patients with thyroid disease, nephrotic syndrome or liver cirrhosis. Postprandial hyperglycemia is a well-known risk factor for atherosclerosis. Although HbA1c reflects mainly mean plasma glucose, GA reflects postprandial plasma glucose or fluctuation of plasma glucose as well as mean plasma glucose. Therefore, GA is a better glycemic indicator than HbA1c in order to prevent the onset and/or suppress the progression of atherosclerosis, one of important chronic diabetic complications.

Oral presentations

O-1

Improvement of substrate specificity of fructosyl peptide oxidase used for measurement of glycated protein

○Atsushi Ichiyanagi[1], Yosuke Masakari[1], Bunta Watanabe[3], Kozo Hirokawa[2], Keiko Gomi[1], Toru Nakatsu[4], Hiroaki Kato[4], Naoki Kajiyama[1]

[1]R&D Div., Kikkoman Corp., [2]Kikkoman Biochemifa Co.,

[3]Inst. Chem. Res., Kyoto Univ., [4]Grad. Sch. Pharm. Sci., Kyoto Univ.

HbA1c as a biomarker for diabetes grows in important with the revision of diagnostic criteria for diabetes in 2010. We have discovered an enzyme, fructosyl peptide oxidase (FPOX), catalyzing the oxidative deglycation of N^{α}-(1-deoxy-D-fructos-1-yl)-L-valyl-L-histidine (F-VH) liberated from the N-terminus of the β-subunits of hemoglobin and developed the novel measurement method of HbA1c using FPOX. In this study, we deduced the amino acid residues which dominate the substrate specificities of FPOXs by comparing crystal structures of FPOXs from *Coniochaeta* (FPOX-CET) and *Eupenicillium* (FPOX-EET), and tried to alter the substrate specificity of FPOX by substituting these amino acid residues with the aim of improving the performance of FPOX. As a result, we obtained the FPOX-CET variant with high substrate specificity and high specific activity by mutating the amino acid residues different among two FPOXs at their substrate binding site.

O-2

Engineering fructosyl peptide oxidase to improve activity toward the fructosyl hexapeptide standard for HbA1c measurement

○Stefano Ferri[1], Yusuke Miyamoto[1], Akane Sakaguchi-Mikami[2], Wakako Tsugawa[1], Koji Sode[1]

[1] Department of Biotechnology, Tokyo University of Agriculture & Technology

[2] School of Biotechnology and Bioscience, Tokyo University of Technology

Adequate metabolic control of blood glucose is important for improving the overall quality of life in diabetes patients as well as to help delay or even prevent the onset of long-term complications of hyperglycemia. Measuring the glycated hemoglobin A1c (HbA1C) levels is the preferred method for assessing long-term control of glycemic levels. The fructosyl hexapeptide fVHLTPE, which is liberated from the amino terminus of HbA1c by digestion with the enzyme endoproteinase Glu-C, has been established as an international standard for the measurement of HbA1c.

Rapid and reproducible enzyme assay systems based on the enzyme fructosyl amino acid oxidase have offered an attractive alternative for conventional clinical tests of glycated proteins. While some of these enzymes have relatively high activity toward small glycated peptides, and are referred to as fructosyl peptide oxidases (FPOXs), none have yet been reported to react with fVHLTPE. We prepared the glycated hexapeptide measurement standard of HbA1c, fVHLTPE, and used it to assay the activity of various FPOXs. No activity was detected with the highly reactive *Coniochaeta* FPOX (FPOX-C); however, the recently discovered FPOX from *Phaeosphaeria nodorum* (PnFPOX) reacted effectively with the hexapeptide. By comparing the structures of these two FPOXs, we identified two loop regions that greatly influence their enzymatic properties. One loop has a strong influence on the ability to bind larger glycated peptides, while the other loop affects catalytic activity. We engineered FPOXs with improved activity towards fVHLTPE by carrying out loop substitutions. Further engineering yielded an FPOX with 17-fold greater dehydrogenase activity against fVHLTPE than wild-type PnFPOX. We are approaching the day when the internationally recognized standard of HbA1c will be directly and conveniently measured by an enzyme-based system.

1) Ferri, S., Kim, S., Tsugawa, W. and Sode, K. (2009) Review of fructosyl amino acid oxidase engineering research: a glimpse into the future of hemoglobin A1c biosensing. *J. Diabetes Sci. Technol.* 3, 585-592.

2) Kim, S., Ferri, S., Tsugawa, W., Mori, K. and Sode, K. (2010) Motif-based search for a novel fructosyl peptide oxidase from genome databases. Biotechnol. Bioeng. 106, 358-366

O-3

A Novel Glycation Inhibitor and Combination Effects of Inhibitors

○Keiichi Kimura[1], Teruyuki Usui[1,3], Hirohito Watanabe[2] and Fumitaka Hayase[1]

[1]Department of Agricultural Chemistry, Faculty of Agriculture, Meiji University,
[2]Department of Life Science, Faculty of Agriculture, Meiji University,
[3]Department of Healthcare Nutrition, Showagakuin Junior College

FAD (Flavin adenine dinucleotide) decomposes Amadori product into amino acid and osone. The osone might be induced protein modification and production of AGEs. In this study, we described novel findings on degradation of fructose-*p*-toluidine and investigated the combination effects of flavins and pyridoxamine. The results indicated that the inhibitory effect of riboflavin was approximately 30 times of FAD. *p*-Toluidine was completely reproduced from fructose-*p*-toluidine by riboflavin with pyridoxamine. Therefore, it was suggested that the osone formed from Amadori product was scavenged by pyridoxamine. Moreover, AGEs level of glycated-protein was in the order of riboflavin < FMN < FAD, and the amount of remaining lysine residue was increased by riboflavin.

O-4

Amino Acid Sequence of Glyceraldehyde-modified Protein

○**Teruyuki Usui[1,2]、Tadashi Yamagishi[2]、Hirohito Watanabe[3]、Fumitaka Hayase[2]**

[1]Department of Healthcare Nutrition, Showagakuin Junior College

[2]Department of Agricultural Chemistry, Meiji University

[3]Department of Life Science, Meiji University

The advanced glycation end products (AGE) were exist in glyceraldehyde-modified protein. Amino acid sequences of the protein were analyzed by LC-MS/MS. Protein (lysozyme, cytochrome C, or ribonuclease A) and glyceraldehyde were incubated in phosphate buffered solution (pH 7.4) at 37℃ for 0-240 h. Glyceraldehyde-modified protein was purified by SDS-PAGE, and the protein was digested by trypsin. Trypsin-treated peptides were analyzed by LC-MS/MS（LTQ Orbitrap）. The result indicated that proteins were modified as adducts and cross-linked structure by glyceraldehyde. The pyridinium-type AGEs were generated in K33, K97, and K116 in the protein. The imidazolinon-type AGEs also were generated in arginine residues.

O-5

Glyoxalase I Ameliorates Glycation and Age-related Endothelial Dysfunction

○Jo A[1], Ohse T[1], Inagi R[1], Ikeda Y[1], Miyata T[2], Takahashi M[3], Nishimatsu H[4], Hirata Y[3], Nangaku M[1]

[1]Division of Nephrology and Endocrinology, [3] Department of Cardiovascular Medicine, [4]Departiment of Urololgy, The University of Tokyo, [2]Tohoku University

Objective: Endothelial dysfunction is a major culprit of cardiovascular diseases, especially in elder people. To investigate how vascular glycation with aging affects endothelial function, we employed the rats systemically overexpressing glyoxalase I (GLO-1), which detoxifies methylglyoxal (MG), a representative advanced glycation end-product (AGE) precursor.
Method: Four groups of rats: young (13-week)/ old (48-week) and wild type/GLO-1 transgenic (WT/ Tg) rats, were examined. GLO-1 activity, glycated protein level of aortic intima, endothelium -dependent and -independent vasorelaxation, and endothelial nitric oxide synthase (eNOS) phosphorylation were measured.
Results: Blood pressure, glucose tolerance, and lipid metabolism were comparable between age-matched WT and Tg. MG-adduct (argpyrimidine) was increased in aortic intima of old WT compared with young, while age-related accumulation of argpyrimidine was significantly reduced in Tg. Tension studies showed attenuation of age-related endothelial dysfunction in Tg compared with WT, while there was no difference in endothelium-independent vasorelaxation between WT and Tg. eNOS phosphorylation on Thr495, which results in decreased eNOS activity, decreased in Tg rats.
Conclusion: The present study revealed that glycation was accelerated in endothelium with aging, and that age-related endothelial dysfunction was attenuated by GLO-1 overexpression, with decreased eNOS phosphorylation on Thr495. It is reported extracellular AGEs are associated with age-related endothelial dysfunction, however, the role of intracellular glycation on endothelial function is not elucidated. Our study is novel as intracellular glycation is suggested to associate with vascular aging. GLO-1 is a potent target for prevention and treatment of age-related endothelial dysfunction and CVD.

O-6

Glucolipotoxicity and inappropriate leptin action induce pancreatic β−cell apoptosis and impair insulin secretion via RAGE induction

○**Dong Han, Yasuhiko Yamamoto, Seiichi Munesue, Hiroshi Yamamoto**

Department of Biochemistry and Molecular Vascular Biology
Kanazawa University Graduate School of Medical Sciences

Type 2 diabetes is a metabolic disorder dramatically increasing in its prevalence worldwide. In the pre-diabetic state, the pancreatic β-cells increase insulin secretion to compensate for insulin resistance with increasing β-cell size and to maintain a normal glucose homeostasis. However, upon exhaustion of β-cells, glucose homeostasis is impaired and overt type 2 diabetes develops. Glucolipotoxicity, which is exerted by free fatty acids (FFA) and prolonged hyperglycemia, is implicated in the pancreatic β-cell failure. However, the underlying molecular and cellular mechanism remains to be defined. We have found that RAGE expression on β-cells only in leptin signaling deficient type 2 diabetic animal models including *ob/ob* and *db/db* mice. We therefore hypothesize that a positive link between insufficient leptin signaling and induction of RAGE expression. In this study, we tried to clarify the links of RAGE expression to leptin action, glucotoxicity, insulin secretion, and apoptosis using mouse pancreatic β-cell line MIN6 *in vitro*. Flow cytometry analysis revealed that palmitate or oleate at 0.2 mM could induce cell surface RAGE expression in MIN6 cells after 24 h exposure. A combination of 12 h pretreatment of 0.2 μg/mL super-active mouse leptin antagonist to block leptin receptor signaling with palmitate or oleate for 24 h could further upregulate RAGE expression in MIN6 cells. When glucose-induced insulin secretion was assayed, the insulin secretion from the MIN6 cells was significantly decreased by exposure to AGE-BSA compared to non-glycated BSA and it was most deteriorated by pretreatment with palmitate with or without the leptin antagonist. Finally, we performed an apoptosis assay by using CellEvent caspase-3/7 green detection reagent and a fluorescent microscope that clearly demonstrated a significant increase in AGE-elicited apoptosis for the MIN6 cells in the case of the pretreatment with palmitate or oleate in combination with the leptin antagonist. These results indicate that elevated FFA with hyperglycemia-mediated AGE formation may cause β-cell damages through induction of RAGE expression due to an insufficient leptin action. Supplementation of sufficient leptin action may be protective against glucolipotoxicity in β-cells in type 2 diabetes.

O-7

The effect of decreasing AGEs using Functional Sweetener Maltitol

○Aya Kuretani[1]、Ryosuke Kawaura[1]、Seiichi Munesue[2]、Yasuhiko Yamamoto[2]、Junya Honda[1]、Hiroshi Yamamoto[2]

[1]Ueno Fine Chemicals Industry, Ltd., [2]Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Sciences

Maltitol is a kind of polyol. It is hydrogenated maltose, and be reduced the carbonyl. Sweetness of maltitol is 80-90%, and its energy value is 1/2 compared with sucrose. Maltitol is not a cause for tooth decay. Therefore maltitol is applicable for health-oriented foods such as sugarless confectionary. This study was aimed to evaluate glycation reactivity using powder maltitol in the case of food manufacturing and a body. Comparison examination of sucrose and maltitol was performed using food model, and fructose and sorbitol were further performed using vivo model. Consequently, in food model with using of egg albumin, milk albumin and milk casein, maltitol decreased 3-DG and fluorescent AGEs. Further in vivo model with using of human serum albumin, gamma globulin and collagen, sorbitol decreased 3-DG, fluorescent AGEs, CML and pentosidine. Moreover, RAGE signaling activity decreased in both models samples. These results suggest it is the substance which is hard to be utilized for a reaction in food and in vivo.

O-8

The Effect of Methylglyoxal Modification on the Chaperone and Anti-apoptotic Functions of Human α-Crystallin

○**Tomoko Oya-Ito[1]、Ram H. Nagaraj[2]、Yuji Naito[1]、Toshikazu Yoshikawa[1]**

[1]Department of Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine、

[2]Department of Ophthalmology and Visual Sciences, Case Western Reserve University

The small heat-shock molecular chaperone protein α-crystallin is a robust anti-apoptotic protein. Immunoprecipitation experiments revealed that methylglyoxal (MGO)-formation occurs in both αA- and αB-crystallin of the human lens. Argpyrimidine was identified at Arg-21 in MGO-modified α-crystallin. The chaperone activity of α-crystallin was enhanced by MGO modification. BioPORTER-mediated transfer of αA- and αB-crystallin into CHO cells resulted in significant protection against hyperthermia-induced apoptosis. This effect was enhanced in MGO-modified αA- and αB-crystallin. ROS generation in apoptotic cells was significantly reduced by transfer of MGO-modified αA- and αB-crystallin. Furthermore, caspase-3 activation was inhibited in MGO-modified α-crystallin transferred cells. Taken together, these results suggest that low cellular concentrations of MGO may help cells to cope with stress by preventing protein aggregation and apoptosis.

O-9

The effect of cocoa Maillard products on high-fat diet-induced fatty liver formation of the rat.

Koichiro Suzuki[1,2], Sachi Hasegawa[1], Fumiko Kimura[1], Kiyotaka Nakagawa[1], Teruo Miyazawa[1]

[1]*Food and Biodynamic Chemistry Laboratory, Graduate School of Agricultural Science, Tohoku University,* [2]*Research Institute, Morinaga and Company, Limited*

Aim of this study: Maillard reaction occurs during food processing and has been recognized as favorable reaction for creating appetite-inducing color and flavor. It was pointed out that diabetic disease might be exaggerated by Maillard reaction products (MRPs). Researches on the effects of MRPs consumption have been studied; however, no conclusion has been reached. Most of previous studies used heated diets to prepare MRPs; therefore, it is difficult to consider these MRPs as daily foods MRPs. In this study, to evaluate the effect of MRPs in daily foods on oxidative stress, model of food MRPs was prepared with or without heat of cocoa. These cocoa were fed to high-fat fed rats, a reported animal model for oxidative stress.

Results: Carboxymethyllysine (CML) and pyrazine in cocoa were measured by LC-MS/MS and GC-MS, respectively. Heated cocoa (cocoa140) contained CML and pyrazine, about 1.4 and 1.3 times higher, respectively, than non-heated cocoa (cocoa0). Wistar rats were fed either a low-fat diet (10% energy as fat), or a high-fat diet (60% energy as fat), a high-fat diet supplemented with 12.5% cocoa0 or cocoa140 for 6 weeks. The hepatic triglyceride level of high-fat diet rats was significantly higher than those of low-fat diet rats, meanwhile both cocoa supplemented rats eliminated the hepatic triglyceride accumulation. This suggested that cocoa has some function for preventing fatty liver formation, regardless of the heat treatment of cocoa. Liver phosphatidylcholine hydroperoxide (PCOOH) concentrations showed significant increase in high-fat diet rats compared with low-fat diet rats, on the other hand, both cocoa supplemented rats did not show further increased PCOOH concentrations in high-fat diet rats. There were no differences in α-tocopherol levels or inflammatory gene transcription levels in the liver by high-fat diet rats and cocoa supplemented rats. The MRPs present in daily food may not interfere the preventive function on fatty liver formation of cocoa, and not accelerate the high-fat diet-induced oxidative stress in vivo.

O-10

Ascorbic acid deficiency in aldehyde reductase knockout mice

○**Motoko Takahashi[1], Satoshi Miyata[2,3], Junichi Fujii[4], Tomoyoshi Soga[5], Naoyuki Taniguchi[6], Yoshio Kuroki[1]**

[1]Sapporo Medical University, [2]Kobe University Graduate School of Medicine, [3]Osaka Koseinenkin Hospital, [4]Yamagata University, [5]Keio University, [6]RIKEN Advanced Science Institute

Aldehyde reductase (AKR1A; EC 1.1.1.2) catalyzes the reduction of various types of aldehydes in an NADPH-dependent manner. We established AKR1A knockout mice and found that the levels of ascorbic acid in livers, kidneys and serum of AKR1A knockout mice were around 5% of those of control mice. The activities of glucuronate reductase and glucuronolactone reductase, which are involved in ascorbic acid biosynthesis, are significantly suppressed in the tissues. We confirmed that AKR1A has activities of both glucuronate reductase and glucuronolactone reductase. Through the introduction of human AKR1A transgene in AKR1A knockout mice, glucuronate reductase activity and glucuronolactone reductase activity are significantly upregulated, and the ascorbic acid concentrations are completely restored. Metabolomic analyses of livers and kidneys of the knockout mice indicated accumulation of D-glucuronic acid and saccharate, an oxidized compound of D-glucuronic acid. Thus, it is suggested that AKR1A is a major enzyme that catalyzes the reduction of both D-glucuronic acid and D-g-glucuronolactone in vivo.

O-11

N（ε- carboxymethyl）lysine (CML) and Myeloperoxidase (MPO) in human carotid atherosclerotic lesion

○Riturou Inoue 1) 2), Noriyuki Sakata 2), Ryoji Nagai 3), Kiyoshi Kazekawa 1)

1) Department of Neurosurgery, Fukuoka University Chikushi Hospital, 2) Department of Pathology, Faculty of Medicine, Fukuoka University and 3) Department of Bioscience, School of agriculture, Tokai University

Our previous study showed that CML was accumulated in advanced lesions of atherosclerosis. In symptomatic patients who underwent carotid artery angioplasty and stenting, CML was significantly increased in debris, compared to asympotmatic patients. In this study, we pathologically examined atheromatous lesions obtained by carotid endarterectomy (CEA), and identified CML and MPO in extracts from these lesions. Material & Methods : We classified the lesions of CEA specimens with AHA (American Heart association) and extracted protein from these lesions. Extracts were subjected to SDS-PAGE and western blot analysis, using anti-CML (6D12) and ant-MPO antibodies. Results: Neither CML nor MPO was detected in non-plaque lesion. CML, but not MPO, was detected in stable plaque lesion. Both CML and MPO were detected in ruptured plaque. CML was detected in the sample of lipid core, but not in that of intima-media and fibrous intimal layer. Conclusions: These findings suggest that CML and MPO may involve in the progression and destabilization of human carotid atherosclerotic lesion.

O-12

Detection of novel methylglyoxal-derived AGE structure in human serum

○Mime Nagai [1], Jun-ichi Shirakawa [1], Yukio Fujiwara [2], Ryoji Nagai [1]

[1]Laboratory of Food and Regulation Biology, Tokai University,

[2]Department of Cell Pathology, Kumamoto University

Methylglyoxal is one of the most reactive dicarbonyls which is generated from the Embden-Meyerhof pathway. Several AGE structures such as Imidazolone and N^{ϵ}-(carboxyethyl)lysine (CEL) have been identified. In the present study, we speculated the presence of carboxyethylated arginine (CEA) and developed its detection system. As results, our monoclonal antibody against CEA significantly recognized MG-modified protein in time dependent manner. Furthermore, CEA was detected both antibody system and liquid chromatography-tandem mass spectrometry in human serum. These results suggested that CEA will be a possible clinical marker for AGE content in human serum.

O-13

Detection of AGEs in serum by LC/MS/MS analysis

Jun-ichi Shirakawa, Mime Nagai, Ryoji Nagai
Laboratory of Food and Regulation Biology, Department of Bioscience,
School of Agriculture, Tokai University

*A*dvanced glycation end-products (AGEs), formed by oxidation and glycation, that accumulates markedly during aging process and the pathogenesis of diabetic complications. However, since the stability and detection procedures of each AGE is different, the development of reliable quantification system for AGEs is required. In the present study, we compared which procedures were suitable for AGEs analyses by LC/MS/MS. Serum sample was hydrolyzed in 6M hydrochloric acid for 18 h then applied to several resins. Our result demonstrated that ion-exchange column would be a suitable for AGEs analyses.

O-14

Distribution of a pair of glycated amino acid metabolizing enzymes, fructosamine 6-kinase and deglycase among bacterial species.

○TSUGAWA,Wakako; KAMIO,Eri; SODE,Koji

Dep. Biotech., Graduate School of Engineering,Tokyo Univ. of Agric. & Technol.

In *Escherichia coli* and *Bacillus subtilis*, fructosamine 6-kinase (FN6K) and fructosamine 6-phosphate deglycase decompose Amadori products. FN6K catalyzes the phosphorylation at the C6 position and produces the stable intermediate fructosamine 6-phosphate. The second enzyme deglycase then catalyses the cleavage of the fructosamine 6-phosphate to glucose 6-phosphate and the corresponding amine. In *E. coli* and *B. subtilis,* the genes encoding the kinase and deglycase are part of an operon. In this study, we will report the deglycases from *Clostridium* species in which we had previously discovered FN6Ks. We also discuss the substrate specificities of these two newly discovered glycated amino acid-metabolizing systems.

Poster session

P-1

Overcoming the heterogeneity of schizophrenia by profiling of carbonyl stress biomarker

○Makoto Arai [1,2], Masanari Itokawa [1], Naila Rabbani [2], Paul J Thornalley [2]

[1] Department of Psychiatry and Behavioral Sciences, Project for Schizophrenia & Affective Disorders Research, Tokyo Metropolitan Institute of Medical Science

[2] CSRI, Warwick Medical School, University of Warwick, University Hospital

Heterogeneity in schizophrenia makes the search for molecular mechanisms much more difficult. Specific biomarkers for schizophrenia have the potential to be useful for identifying patients at risk of developing the disease and distinguish biologically meaningful subgroups within the disease. The aim of this study is to overcome the heterogeneity of schizophrenia by personalized profiling of protein damage biomarkers (Omics-based Personalized Medicine). Carbonyl stress is a new target of medication for schizophrenia without neurotransmitter based concept of therapeutics. In particular, the markedly high pentosidine level in schizophrenic patients with low vitamin B6 level suggests that pyridoxamine may prove clinically useful.

Further studies are needed to verify the effect of antipsychotic medication on elevated plasma pentosidine and characterize clinical features of schizophrenia with carbonyl stress as well as elucidating precise molecular mechanisms of pentosidine in central nerve systems of the disease. In my talk, I would like to provide a brief overview of omics-based medicine of the disease, covering an *in vivo* clinical trial using pyridoxamine and an *in vitro* biological study in progress.

P-2

Methylglyoxal Induces Oxidative Injury in Dorsal Root Ganglion

○Nozomi Taki, Hiromitsu Oozeki, Michiru Kamizono, Jun Kotera
Advanced Medical Research Laboratories, Mitsubishi Tanabe Pharma Corporation

Carbonyl stress has been shown to be involved in diabetes and diabetic complication. Hyperglycemia increases a highly reactive dicarbonyl metabolite, methylglyoxal (MG), causing carbonyl stress in cells. Modification of proteins by MG changes their functions and generates advanced glycation end products (AGEs). These products generated by MG result in induction of oxidative stress and inflammation. Recent papers have reported that MG accumulation is associated with development of diabetic neuropathy. However it is not clear whether MG directly affects in dorsal root ganglion (DRG), which mediates central sensitization caused by peripheral mechanical stimulus in hyperalgesia. Here, we demonstrated that MG induced apoptosis and decreased glutathione in cultured DRG and PC12 cells. Expression of antioxidant enzyme genes such as heme oxygenase 1 and NADPH quinone oxidoreductase 1 were induced by MG treatment to prevent oxidative stress. MG also caused axonal damage in DRG neurons. All of these effects by MG were inhibited by N-acetylcysteine (NAC) which is known as an antioxidant and a MG scavenger. These results suggest that MG induces neurotoxicity through oxidative stress in DRG and play a pivotal role in diabetic neuropathy.

P-3

The novel mechanism of transcriptional activation of Carbohydrate Response Element-binding Protein (ChREBP)

○Haruhiko Sakiyama, Noriko Fujiwara, Hironobu Eguchi, Daisaku Yoshihara, Keiichiro Suzuki

Department of Biochemistry, Hyogo College of Medicine

The carbohydrate response element-binding protein (ChREBP) functions as a transcription factor in mediating the glucose-activated gene expression of multiple liver enzymes, which are responsible for converting excess carbohydrate to storage fat. ChREBP is translocated into the nucleus in response to high glucose levels, and then up-regulates transcriptional activity. Because high glucose conditions induce the glycosylation of cellular proteins, the effect of *O*-linked GlcNAc modification on ChREBP functions was examined. Treatment with an *O*-GlcNAcase inhibitor (PUGNAc), which increases the *O*-linked GlcNAc modification of cellular proteins, caused an increase in the glucose response of ChREBP. In contrast, treatment with a glutamine fructose amidotransferase inhibitor (DON) completely blocked the glucose response of ChREBP. These results suggest that the *O*-linked glycosylation of ChREBP itself or other proteins that regulate ChREBP is essential for the production of functional ChREBP.

P-4

Reactivity of All-trans-retinal and various amines, and Subsequent Photosensityzed Model Reaction of Vitamin A

Kyozo Suyama, Toshio Miyata

Center for translational and Advanced Research, Tohoku University, School of Medicine

The retinal pigments that accumulate in epithelial cells have been implicated in the etiology of macular degeneration. The major pigment is the A2E, an ethanolamine pyridinium bisretinoid. It have been reported that light-exposed A2E epithelium exhibits a propensity for apoptosis with blue light by the singlet oxygen oxidation. We demonstrated the reaction between all-trans-retinal(atRet) and various biological amines, and oxidation by single oxygen model reaction of VA. The reaction of atRet and amines were carried out under the same reaction system of A2E synthesis in 5% acetic acid/methanol. Retinol palmitate was irradiated under the natural sunlight. (Results) The structure of amine was greatly affected on the reactivity for atRet, and there were some amines having inhibitory effect on the reaction. Photosensitized reaction on VA gave various volatile degradation products such as ionone.

P-5

Vascular Effects of Imidazolones, L-Arginine–derived Advanced Glycation End-products (AGEs)

○Naoto Koyama,[1] Mandai Yamada,[2] Chika Yoshida[1] and Kenzo Nomura[1]
[1]Institute for Innovation, [2]Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co., Inc.

Aim of this study: To examine *in vitro* and *in vivo* vascular effects of the chemically-defined AGEs, L-arginine-derived imidazolones.

Results: Total of seven 5-hydro-, or 5-methylimidazolones were structurally identified. All of them relaxed rat aortic rings. Because the vasodilative activity was shown to be endothelium-dependent, PI3K inhibitor-sensitive, and accompanied by eNOS-Ser1177 phosphorylation, eNOS stimulation via PI3K/Akt pathway was suggested for their mode of action. Orally-administered MG-H1 (methylglyoxal-derived hydroimidazolone isomer1) was rapidly absorbed into the circulation of mice, and significantly decreased blood pressure in phenylephrine-induced hypertensive rats. Moreover, when MG-H1 was simultaneously administered with L-Arg, the antihypertensive effect was significantly greater than when these compounds were administered separately.

Conclusion: Dietary L-Arg-derived imidazolones may have some impact on cardiovascular health.

P-6

Screening of novel fructosamine 6-kinase from protein database

○KAMIO,Eri; TSUGAWA,Wakako; SODE,Koji

Dep. Biotech., Graduate School of Engineering,Tokyo Univ. of Agric. & Technol.

[Objective]

Fructosamine6-kinase (FN6K) is enzyme which catalyzies ATP dependent phosphorylation of fructosyl amino acid. Therefore, it is expected as enzyme measuring glycated protein.FN6K is soluble protein which is isolated from *Escherichia coli* and *Bacillus subtilis.* In this work,we report screening novel FN6K from genomic database.

[Method and results]

First, we searched sequences having high identity with FN6K from *E.coli* from genomic database. And then ,we selected 2 species from *Clostridium* having 30 %~70 % identity with FN6K from *E.coli.* Next, we did cloning of these genes and expression of them using *E.coli..* As a result, it was confirmed that these 2 candidates proteins have FN6K activity in soluble fraction and have broad substrate specificity compared with FN6K from *E.coli.*

P-7

An Amperometric Sensor based on a Soluble Molecularly Imprinted Catalyst for Fructosyl Valine

○Tomohiko YAMAZAKI [1] and Koji SODE [2]

[1] World Premier International (WPI) Research Center for International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS) [2] Graduate School of Engineering, Tokyo University of Agriculture and Technology

An amperometric sensor based on a soluble molecularly imprinted catalyst (MIC) has been developed for the detection of fructosyl amine compounds. A soluble MIC containing water-soluble functional monomers, an imidazole catalyst, and small amounts of a hydrophilic cross-linker is developed and used as a fructosyl amine oxidase mimic and for amperometric sensor construction. Fructosyl valine (Fru-val), a model compound of glycated hemoglobin, HbA1c, is used as the template. The MIC specifically oxidizes Fru-val in the presence of 1-methoxyphenazine methosulfate (electron acceptor) and reacts with the glycated peptide, fructosyl-valinehistidine sequence at the N-terminal of the β-globin in HbA1C. We also fabricated an amperometric biosensor for Fru-val determination by immobilization of the soluble MIC onto an Au electrode. The amperometric MIC-based sensor showed higher sensitivity to Fru-val than to Fru-ε-lys, the competitor in HbA1c detection.

P-8

Development of immunoassay devices for HbA_{1c} based on ion-exchange chromatography

○Takumi Shimada, Kojiro Izawa, Tadashi Matsunaga, Tsuyoshi Tanaka, Tomoko Yoshino
Institute of Engineering, Tokyo University of Agriculture and Technology.

An on-chip type cation-exchange chromatography system with electrochemical detection of HbA_{1c}, which is one of the most important diabetes marker protein, was developed using ferrocene-conjugated anti-human hemoglobin (Hb) monoclonal antibody (FcAb). The FcAb was used as an electrochemical probe for the detection of each Hb. The separation conditions of HbA_{1c} in blood calibrator samples from other Hbs, e.g. HbA_0, HbA_{1a} or HbA_{1b}, were optimized using the on-chip type system. The electrochemical oxidation current from FcAb reacting with each Hb was measured at 350 mV (vs. Ag/AgCl). Hbs including HbA_{1a} and HbA_{1b}, HbA_{1c} and HbA_0 fractions were eluted in this order. A linear relationship between HbA_{1c} levels and electrochemical oxidation currents was obtained in the range from 4.0% to 12.6% HbA_{1c}. Furthermore, a good correlation was obtained between KO500 method (HPLC) and our proposed method. These results indicate that the on-chip type system with electrochemical detection can be applied to a novel POCT device for rapid and precise detection of HbA_{1c}.

P-9

Cloning and characterization of SocR as a novel glycated products-responsive transcriptional regulator

○Shuichi Oya, Isao Karube, Akane Sakaguchi-Mikami
[1]School of Biotechnology and Bioscience, Tokyo University of Technology

Monitoring systems for intracellular glycated products (GPs) have been expected to contribute to the understanding of detail mechanisms for intracellular glycation and for pathogenesis of GPs-affected disease. We previously demonstrated the construction of the intracellular glucose monitoring system by employing Lac repressor (LacI)-glucose binding protein chimeric protein as a transcriptional regulator of a reporter assay. A LacI-like protein, SocR, is encoded upstream of *Agrobacterium tumefaciens*'s α-fructosyl amino acid (α-FA) dependent operon which consists of α-FA specific proteins. In this paper, we report the cloning and characterization of SocR as a novel GPs responsive regulator towards the development intracellular monitoring systems for glycation.

Local Committee of 22nd JMARS annual meeting
Koji Sode (Tokyo University of Agriculture & Technology)
Wakako Tsugawa (Tokyo University of Agriculture & Technology)
Akane Mikami (Tokyo university of Technology)
Katsuhiro Kojima (Tokyo University of Agriculture & Technology)
Koichi Abe (Tokyo University of Agriculture & Technology)
jmars22@cc.tuat.ac.jp
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