《Research Note》

Incorporation of Glycated-Tryptophan and -Valine into Various Cells Derived from Chicken Embryos

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Avian species including chickens are known to be hyperglycemic animals. Hyperglycemia promotes the glycation which at first forms Amadori products undergoing further complex reaction to form advanced glycation end products (AGEs). Our previous study revealed that AGEs derived from glucose and amino acids were predominantly incorporated into spleen, kidney and liver. However, it has not been elucidated whether Amadori products (glycated amino acids) can also be incorporated into cells or not. Therefore, in the present study, radioactive glycated-tryptophan and -valine were prepared and the incorporation of these glycated amino acids into various chicken embryonic cells was studied. Various embryonic cells prepared from muscle, liver, spleen and kidney of chicken embryos were incubated in Medium 199 supplemented with ¹⁴C-labeled glycated-tryptophan or -valine. After incubation, embryonic cells were well-rinsed and then the radioactivity incorporated into cells was measured. It was revealed that both glycated amino acids were incorporated into embryonic cells derived from muscle, liver, spleen and kidney. In muscular cells, the incorporation of glycated-tryptophan was higher than that of glycated-valine. On the other hand, in embryonic cells derived from liver and kidney, the amount of glycated-tryptophan incorporated into cells was almost the same to that of glycated-valine. In conclusion, it was supposed that both glycated-tryptophan and -valine could be incorporated into various cells derived from muscle, liver, spleen and kidney of chicken embryos and that the incorporation might have the organ specificity.

Key words: cellular incorporation, chicken embryo, glycation, tryptophan, valine

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Introduction

The aves including chickens are known to be hyperglycemic animals. In healthy persons, fasting blood glucose concentration is maintained under 110 mg/dl. Meanwhile, chicken blood glucose is over 200 mg/dl (Hazelwood and Lorenz, 1959). Hyperglycemia promotes the glycation which is a non-enzymatic reaction causing dehydrating condensation between the carbonyl group of glucose and the amino group of amino acids. Because of high body temperature in avian species, glycation, so-called Maillard reaction (Maillard, 1912) or amino-carbonyl reaction, is easily proceeded compared to mammals (Klandorf *et al.*, 1995; Iqbal *et al.*, 1999). Glycation forms Amadori product which undergoes further complex reaction to form advanced glycation end products (AGEs).

In the previous study, radiolabeled AGEs prepared by

reacting ¹⁴C-glucose and 20 amino acids mixture were intravenously administrated to investigate the tissue distribution of AGEs in chickens. This study revealed that radio-labeled AGEs were predominantly incorporated into spleen, kidney and liver (Kita, 2014). However, it has not been elucidated whether Amadori products (glycated amino acids), which are the precursors of AGEs, can be also incorporated into cells or not. Previously, we could successfully synthesize Amadori products from glucose and tryptophan, and measured the plasma concentrations of glycated-tryptophan (Kita *et al.*, 2013; Makino *et al.*, 2015). Furthermore, we have been able to synthesize glycated-valine (Takahashi and Kita, 2016). In the present study, therefore, radioactive glycated-tryptophan and -valine were prepared to investigate their incorporation into various chicken embryonic cells.

Materials and Methods

Preparation of Radioactive Glycated Amino Acids

The radioactive amino acids were prepared by incubating amino acid solution with radioactive glucose at 37°C for 7 days. The 19mM non-radioactive D-glucose and 1mM radioactive glucose solution containing U-¹⁴C-D-glucose

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(3.7 MBq/ml; Moravex Biochemicals, Inc., CA, USA) were added into 20 mM tryptophan or valine solution. Final concentrations of amino acids and D-glucose were 10 mM each. After 7 days of incubation, unbound radioactive or non-radioactive glucose were removed by cation-exchange resin (Dowex 50W-X8). The radioactivity of glycated-tryptophan and -valine were determined using a liquid scintillation counter (LSC-1500, Aloka Ltd., Tokyo, Japan).

Preparation of Cells from Various Tissues of Chicken Embryos

Thirty fertilized eggs of Single Comb White Leghorn chickens were purchased from a local hatchery (Koiwai Farm Co., Ltd, Shizukuishi, Iwate, Japan). Embryonic cells from various tissues were prepared as described previously (Kita and Makino, 2014). Briefly, fertilized eggs were incubated for 19 days, and embryos were taken from eggs. After decapitated, breast muscles, liver, spleen and kidney were removed and minced finely with scissors. Minced tissues were gently digested using 0.25% (w/v) trypsin, pipetted several times and passed through the gauze to remove the crumble of tissues. Cells were seeded in a Type-1 collagen-coated 48-well plate (Corning, Bedford, MA, USA) with Medium 199 including 2.5 µg/ml amphotericin, 100 units-100 µg/ml penicillin-streptomycin, 50 µg/ml gentamycin and 10% fetal calf serum (FCS) and incubated at 37°C in 5% CO₂/95% air (v/v). All reagents except penicillin-streptomycin for preparation of various tissue cells were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Penicillin-streptomycin was purchased from Biological Industries Ltd. (Kibbutz Beit Haemek, Israel).

Measurement of Radioactivity of Glycated Amino Acids Incorporated into Cells

After overnight incubation of cells, the culture medium was drawn and added with fresh medium including glycated-tryptophan or -valine. Media were prepared from Medium 199 including 10% FCS and 0 or 60 Bq/ml of glycated amino acids. After further 1 day of incubation, the medium was drawn away and cells were rinsed 3 times with ice-cold Medium 199. It was confirmed that the rinsed medium had no radioactivity. After removing Medium 199, 400 µl of 0.5 % (w/v) NaOH/0.1% (v/v) Triton X-100 was added into the well and incubated at room temperature for 30 min. After dissolving cells by pipetting, the radioactivity in NaOH/Triton X-100 solution was measured using a liquid scintillation counter as an index of incorporation of glycated amino acids.

Statistical Analysis

Statistical analysis of data was performed by two-way ANOVA using the General Linear Model Procedures of SAS (SAS/STAT version 9.4) (SAS Institute, 2012). The main effects were additives (control, glycated-tryptophan and glycated valine) and tissues (muscle, spleen, kidney and liver). Differences between means were considered to be significant at $P < 0.05$.

Results and Discussion

As represented in Fig. 1, both glycated-tryptophan and

-valine were incorporated into embryonic cells derived from liver, spleen and kidney. A large amount of glycated-tryptophan was incorporated into muscle cells compared to other tissue cells, which suggested that the incorporation of glycated amino acids (Amadori products) might have the tissue specificity.

As Kita (2014) revealed that AGEs derived from glucose and amino acids were predominantly accumulated in liver, spleen and kidney of chickens. This report was in good agreement with results observed in the present study. These observation would suggest that both Amadori products and AGEs are easily incorporated into these tissues, liver, spleen and kidney, regardless of quite difference in chemical structure between Amadori products and AGEs.

It was reported that several types of specific cell surface proteins which can bind to AGEs. These types of proteins are called by RAGE (receptor of AGEs) which is well known as a key molecule to mediate cell signaling resulting in diabetic complications (Brownlee, 2001). There reports suggested that AGEs are generated extracellularly from Amadori products. On the other hand, the incorporation of Amadori products into various cells observed in the present study suggested that AGEs could be formed intracellularly from Amadori products. These results suggested that AGEs would be formed not only intracellularly but also extracellularly from Amadori products.

Fig. 1 also showed an interaction between additives and tissues in the incorporation of glycated amino acids. In muscular cells, the incorporation of glycated-tryptophan was significantly higher than that of glycated-valine. Furthermore, the incorporation of glycated-tryptophan into muscular cells was significantly higher than those into embryonic cells derived from liver, spleen and kidney, which was inconsistent with the previous study in which the incorporation of

Fig. 1. The incorporation of glycated-tryptophan and -valine into various cells from the chicken embryo tissues. ^{a-d} Means with different superscript letters within the same additive are significantly different at $P < 0.05$. ^{x-z} Means with different superscript letters within the same tissue are significantly different at $P < 0.05$. Values are means $\pm$ SE. $n = 6$.

glycated-tryptophan into muscles was not high compared to liver, spleen and kidney (Kita, 2014). This inconsistency might be resulted from the ontogeny of muscle development. The physiological and biochemical feature in muscles was quite different between embryonic and post-hatch periods. For instance, although the muscle in embryonic stage was the main site to express the gene of insulin-like growth factor-I (IGF-I), which is one of growth factors stimulating cell and tissue growth directly, after hatching the muscle was changed to be one of main target tissues for IGF-I (Kita *et al.*, 2000; 2002). But this issue is still unsolved and should be elucidated in the future.

In conclusion, it was suggested that both glycated-tryptophan and -valine could be incorporated into various cells derived from muscle, liver, spleen and kidney of chicken embryos. But, the mechanism of the incorporation of glycated amino acids has not been clarified yet. The further investigation should be conducted in the future.

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References

- Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414: 813–820. 2001.
- Hazelwood RL and Lorenz FW. Effects of fasting and insulin on carbohydrate metabolism of the domestic fowl. *American Journal of Physiology*, 197: 47–51. 1959.
- Iqbal M, Probert LL, Alhumadi NH and Klandorf H. Protein Glycosylation and advanced glycosylated endproducts (AGEs) accumulation: An avian solution? *Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 54: B171–B176. 1999.
- Kita K, Noda C, Miki K, Kino H and Okumura J. Relationship of IGF-I mRNA levels to tissue development in chicken embryos of different strains. *Asian-Australasian Journal of Animal Sciences*, 13: 1653–1658. 2000.
- Kita K, Nagao K, Taneda N, Inagaki Y, Hirano K, Shibata T, M. Aman Yaman, Conlon MA and Okumura J. Insulin-like growth factor binding protein-2 gene expression can be regulated by diet manipulation in several tissues of young chickens. *Journal of Nutrition*, 132: 145–151. 2002.
- Kita K, Kawashima Y, Makino R, Namao T, Ogawa S, Muraoka H and Fujimura S. Detection of two types of glycated tryptophan compounds in the plasma of chickens fed tryptophan excess diets. *Journal of Poultry Science*, 50: 138–142. 2013.
- Kita K. The spleen accumulates advanced glycation end products in the chicken: tissue comparison made with rat. *Poultry Science*, 93: 429–433. 2014.
- Kita K and Makino R. Influence of valine analogues on protein synthesis of chicken embryo myoblasts. *Journal of Poultry Science*, 51: 191–194. 2014.
- Klandorf H, Holt SB, McGowan JA, Pinchasov Y, Deyette D and Peterson RA. Hyperglycemia and non-enzymatic glycation of serum and tissue proteins in chickens. *Comparative Biochemistry and Physiology. Part C, Pharmacology, Toxicology and Endocrinology*, 110: 215–220. 1995.
- Maillard LC. Action of amino acids on sugars. Formation of melanoidins in a methodical way. *Compte-Rendu de l'Academie des Science*, 154: 66–68. 1912.
- Makino R, Kawashima Y, Kajita Y, Namao T, Ogawa S, Muraoka H, Fujimura S. and Kita, K. Glycated tryptophan in the plasma of chickens fed tryptophan-excess diets. *Journal of Poultry Science*, 52: 23–27. 2015.
- SAS Institute. SAS/STAT User's Guide. 9th ed. SAS Institute. North Carolina. 2012.
- Takahashi N and Kita K. Fructosyl-valine orally administrated to chickens is incorporated from gastrointestinal tract. *Journal of Poultry Science*, 53: 153–155. 2016.