128. Amphibian Blood Group Substances. III

Biosynthesis of Uridine Diphosphate Galactose in Bull-frog's Livers and Stomachs

By Shigeru YAMAMOTO and Shoei ISEKI

Department of Legal Medicine, School of Medicine,
Gunma University, Maebashi

(Comm. by Tanemoto FURUHATA, M. J. A., June 12, 1971)

Biosynthesis of polysaccharides including blood group substances generally depends on sequential addition of component sugars which are donated from corresponding sugar nucleotides. In fact, uridine diphosphate galactose (UDP-galactose) has been served as a galactosyl donor on biosynthesis of blood group B substance using enzyme preparations derived from gastric mucosa or submaxillary gland in human, baboon, and rabbits,¹⁾⁻⁴⁾ and from human milk,⁵⁾ all with blood group B character.

Present work will describe probability that UDP-galactose participates in as a galactosyl donor as a result of accumulation or formation of the sugar nucleotide in livers or gastric mucosa in bull-frogs by applying ¹⁴C-galactose.

Material and methods. 1. *In vivo incorporation of ¹⁴C-galactose.* Saline-diluted D-galactose-1-¹⁴C (The Radiochemical Centre, CFA-435, specific activity of 55.7 mCi/mM) was injected into the heart of vivid bull-frogs in an amount of 17 μ Ci per frog. Each five frog's livers and stomachs were cut out at 5, 10, 20, 40, 80, and 160 minutes after injection of the ¹⁴C-galactose. The same amount of the isotope was injected into five frogs anesthetized with ether as a control.

2. *In vitro incorporation of ¹⁴C-galactose.* Wet weight of 66 g of the gastric mucosa from fourty frogs was homogenized in ice by a glass homogenizer with 200 ml of 0.35 M sucrose—pH 6.8, 0.05 M tris-HCl buffer. The mucosal homogenates (260 ml) were divided into five portions. Each portion was incubated at 37°C with 100 μ Ci of D-galactose-1-¹⁴C, same as used in the above. At 0, 5, 10, 20, and 40 minutes after incubation, four volume of boiled 90% ethyl alcohol was added into every portion, which was further boiled for 5 minutes, followed by chilling in ice.

Abbreviations used: UDP, uridine diphosphate; GDP, guanosine diphosphate; ADP, adenosine diphosphate.

3. *Isolation of sugar nucleotides.* Hepatic nucleotides were extracted and isolated by the method of O'Brien and Neufeld,⁶⁾ with slight modification by repeated paper chromatography with pH 7.5 and 3.8 of ethyl alcohol—1.0 or 0.5 M ammonium acetate buffer on Whatman 3MM. Gastric nucleotides were also isolated by the same methods used for the hepatic nucleotides except pretreatment described below. The incubation mixture of the gastric homogenates was rehomogenized and centrifuged off by $10,000\times g$ for 15 minutes at 0°C . The residue resulted was washed twice with cold 80% ethyl alcohol. The supernatant and washings were combined and concentrated *in vacuo*. The gastric nucleotides were isolated from the concentrates.

4. *Component analysis.* Nucleotides on paper chromatograms were detected under irradiation of UV lamp and by molybdic acid method for phosphorus esters.⁷⁾ Sugars in nucleotides were identified and determined by the methods stated in the preceding paper.⁸⁾ Content of uridine was calculated using molar extinction coefficient of 10.1×10^3 at $262\text{ m}\mu$. Total phosphate was measured by King's method.⁹⁾ α -Galactosyl residue was assayed by α -D-galactosidase partially purified from *Clostridium sporogenes* Maebashi.¹⁰⁾

5. *Measurement of radio isotope.* ^{14}C of labeled nucleotide solution was measured by a liquid scintillation counter (Tricarb) in a solvent system of dioxane, containing PPO, dimethyl POPOP, naphthalene, and ethylene glycol. ^{14}C of nucleotides on paper was measured by a radio paper scanner (Aloka) or the liquid scintillation counter after placing a piece of paper in a solvent system of toluene, containing PPO and dimethyl POPOP.

Results. 1. *In vivo* incorporation of ^{14}C -galactose into hepatic nucleotides. Paper chromatography with two solvent systems showed various kinds of nucleotides present in bull-frog's livers. That is, UDP-galactose, UDP-glucose, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, and GDP-mannose were identified as a member of sugar nucleotides, and in addition, adenosine monophosphate, inosine monophosphate, thymidine diphosphate, ADP, GDP, guanosine triphosphate, cytidine triphosphate, adenosine triphosphate, and uridine triphosphate, as nucleotides. Radioactivities were found on only two spots which corresponded to UDP-galactose and UDP-glucose, respectively, on chromatographic mobilities. The whole area with radioactivities was extracted with water and hydrolyzed in the presence of HCl. ^{14}C -Labeled sugars of the hydrolyzates were identified to be galactose and glucose. Side by side, UDP-galactose and UDP-glucose were completely separated from each other by paper chro-

matography using two sorts of buffers. The UDP-galactose isolated showed the same absorption spectrum as UDP-galactose purchased from Sigma and gave the maximum absorption at $262\text{ m}\mu$ at pH 7.0. The molar ratio of uridine : total phosphate : galactose in the isolated one is 1 : 2.03 : 1.06, approximating to the theoretical molar ratio of 1 : 2 : 1 in authentic UDP-galactose. Accordingly, qualitative and quantitative analyses indicate one of the ^{14}C -labeled nucleotides to be UDP-galactose. Likewise, another radio-active nucleotide was confirmed to be UDP-glucose on the basis of correspondence to chromatographic behavior and chemical composition.

On the other hand, velocity of biosynthesis of UDP-galactose and -glucose was investigated in pursuit of radioactivities in the hepatic nucleotides after injection of ^{14}C -galactose into the frog's heart. Because it is uneasy to quantitatively separate UDP-galactose from

Fig. 1. ^{14}C incorporation into nucleotide fractions from ^{14}C -galactose.

-X-----X-: ^{14}C -UDP-Glc/5 frog's livers, *in vivo*.

-○-----○-: ^{14}C -UDP-Gal/5 frog's livers, *in vivo*.

-□-----□-: ^{14}C -UDP-hexoses/8 frog's gastric homogenates, *in vitro*.

UDP-glucose, mutual radioactivity was calculated by radioactivity ratio of ^{14}C -galactose to ^{14}C -glucose on paper chromatograms on which the hydrolyzates of the combined UDP-galactose and UDP-glucose isolated at a given time were subjected.

Fig. 1 indicates that UDP-galactose as well as UDP-glucose is promptly synthesized in the frog's livers. As 6.5×10^7 cpm of ^{14}C -galactose was injected per five frogs, incorporation rate of ^{14}C into the two sugar nucleotides was 32.8% after 10 minutes and 33.3% after 20 minutes, the maximum incorporation lying at 10 to 20 minutes after injection of the galactose. In addition, we can see that the amount of UDP-glucose is always higher than that of UDP-galactose at any time set and that the amount of both of them decreases gradually as time elapses longer.

2. *Biosynthesis of UDP-galactose by gastric mucosal homogenates.* Although injection of ^{14}C -galactose resulted in synthesis of two kinds of UDP-sugars in the livers, it did also in incorporation of the radioactivities into the gastric blood group fractions, with rate higher than into hepatic nucleotides.¹¹⁾ In this regard, a doubt is graved that UDP-galactose, supposed to be α -galactosyl donor on biosynthesis of gastric B character may be formed in the gastric mucosa rather than in the livers. Of course, it does not deny that the livers function as a chief organ to synthesize and supply varieties of important nucleotides to the organs which require them. Therefore, incubation of the gastric mucosa with ^{14}C -galactose was carried out and isolation of ^{14}C -labeled nucleotides was tried.

Yield of nucleotides from the gastric homogenates was less than

Fig. 2. Radio paper chromatogram of gastric nucleotide fraction.

Sample: Nucleotide fraction extracted from incubation mixture for 10 min.

Solvent: pH 7.5, ethyl alcohol—1.0 M ammonium acetate.

that from the livers. Paper chromatography showed that all nucleotides found in the livers and an additional nucleotide, guanosine monophosphate were also present in the gastric mucosa. Fig. 2 still more indicates that the radioactivities are distributed on two major spots corresponding to UDP-galactose and UDP-glucose, as well as a minor spot, UDP-N-acetylhexosamine(s). The two nucleotides with high radioactivities were extracted from the paper, purified, and analyzed on chemical composition. Uridine, phosphate, and galactose were found in the one of these hydrolyzates, and also uridine, phosphate, and glucose, in the other. It points out that UDP-galactose and -glucose are main reaction products in the presence of bull-frog's gastric mucosa. As seen also in Fig. 1, biosynthesis of UDP-hexoses rapidly proceeds, reaching the maximum within 20 minutes after incubation. The amount of these gastric nucleotides holds constant from 20 minutes and the accumulation curve is dissimilar to that of UDP-galactose or UDP-glucose formed *in vivo* in the livers. Though it is conceivable that such a discrepancy is partly due to the difference of experimental conditions, *in vivo* and *in vitro*, it is preferable to consider that nucleotides formed *in vivo* in livers are doomed to reduce under normal condition by suffering an attack of corresponding hydrolases or by transportation into the other organs. Setting aside the question, bull-frog's gastric mucosa are confirmed to furnish potent enzyme systems to form UDP-galactose as well as UDP-glucose from galactose, as do the livers.

Discussion. Biosynthesis of galactose-linked nucleotide was investigated in bull-frogs by means of introducing ^{14}C -galactose. *In vivo* and *in vitro* experiments showed that both UDP-galactose and UDP-glucose were rapidly synthesized in the livers and gastric mucosa. However, GDP- and ADP-galactose which are nucleotides associated with galactose seem to be not formed or reserved in these organs. In bull-frog's livers and gastric mucosa, UDP-galactose is considered to be formed from galactose by galactokinase and galactose-1-phosphate uridylyltransferase, or by uridine diphosphogalactose pyrophosphorylase, too, as generally recognized.¹²⁾

Conclusion. Various kinds of nucleotides including UDP-galactose, UDP-glucose, GDP-mannose, UDP-N-acetylhexosamines are found to be present in livers and gastric mucosa of bull-frogs. Incorporation of ^{14}C -galactose into the heart resulted in accumulation of ^{14}C -UDP-galactose and ^{14}C -UDP-glucose in the livers. In addition, the two UDP-hexoses are also rapidly synthesized in the incubation of the gastric mucosa with galactose so that it is recognized that very active enzyme system responsible for formation of UDP-galactose

and UDP-glucose is inherent in both livers and gastric mucosa of bull-frogs.

References

- 1) Zinderman, D., Gompertz, S., Smith, Z. G., and Watkins, W. M.: Glycosyl-transferases in mammalian gastric mucosal linings. *Biochem. Biophys. Res. Commun.*, **29**(1), 56-61 (1967).
- 2) Race, C., Zinderman, D., and Watkins, W. M.: An α -D-galactosyl transferase associated with the blood group B character. *Biochem. J.*, **107**(5), 733-735 (1968).
- 3) Race, C., and Watkins, W. M.: Properties of an α -D-galactosyl transferase in human tissues obtained from blood group B donors. *Biochem. J.*, **114**(4), 86 p (1969).
- 4) Shenkel-Brunner, H., and Tuppy, H.: Enzymes from human gastric mucosa conferring blood-group A and B specificities upon erythrocytes. *Eur. J. Biochem.*, **17**(2), 218-222 (1970).
- 5) Kobata, A., Grollman E. F., and Ginsburg, V.: An enzymatic basis for blood type B in human. *Biochem. Biophys. Res. Commun.*, **32**(2), 272-277 (1968).
- 6) O'Brien, P. J., and Neufeld, E. F.: A rapid procedure for the preparation of small quantities of uridine diphosphate-N-[14 C] acetylhexosamine. *Biochim. Biophys. Acta*, **83**(3), 352-354 (1964).
- 7) Burrows, S., Grylls, F. S. M., and Harrison, J. S.: Paper chromatography of phosphoric esters. *Nature*, **170**(4332), 800-801 (1952).
- 8) Yamamoto, S., and Iseki, S.: Amphibian blood group substances. II. Chemical components of gastric blood group substances from *Xenopus* and *Rana*. *Proc. Japan Acad.*, **46**(2), 191-196 (1970).
- 9) Lindberg, O., and Ernster, L.: in *Methods of Biochemical Analysis*, Vol. 3 (edited by Glick, D.). Interscience Publishers, New York and London, 1-22 (1956).
- 10) Yamamoto, S., Fujisawa, K., and Iseki, S.: Chemical effects of purified A- and B-decomposing enzymes on blood group A and B substances. *Gunma J. Med. Sci.*, **11**(4), 248-258 (1962).
- 11) Yamamoto, S., and Iseki, S.: Amphibian blood group substances. IV. Biosynthesis of blood group B substance in bull-frog's gastric mucosa. *Proc. Japan Acad.*, **47**(6), 573-578 (1971).
- 12) Axelrod, B.: in *Metabolic Pathways*. Vol. 1, edited by Greenberg, D. M., Academic Press, New York and London, 296-298 (1967).