**CHARACTERISTICS OF POLYGLUCOSE PARTICLES NEWLY-
SYNTHESIZED BY THE PHOSPHORYLASE-BRANCHING
GLYCOSYLTRANSFERASE SYSTEM IN VARIOUS
TISSUE CELLS UNDER HISTOCHEMICAL
CONDITIONS**

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Since the original method was reported by Takeuchi and Kuriaki (28), it has been known that the histochemical demonstration of phosphorylase activity with iodine under the light microscope produces various colors according to the kind of tissue cells. On the other hand, electron microscopic observation of polyglucose synthesized from glucose-1-phosphate by phosphorylase activity was first made by Sasaki and Takeuchi in human muscle fibers (18). Since then, intracellular polyglucose synthesis has been studied in several kinds of tissue cells under the electron microscope (2, 12, 13, 14, 15, 17, 26, 29, 30, 31, 33). Takeuchi and his co-workers found that newly-formed polyglucose particles synthesized from glucose 1-phosphate (G1-P) by the phosphorylase and branching glycosyltransferase system (PBG-system) under histochemical conditions varied in size, shape and electron-density according to the kind of tissue cells and that they were different from endogenous glycogen particles. As reported previously, Miyayama (12, 14) demonstrated that newly formed polyglucose particles synthesized from G1-P by the PBG system in white muscle fibers of the rat were stained blue with iodine and were either amorphous aggregates or much smaller in size than endogenous glycogen particles. This finding coincided with Takeuchi and Sasaki's finding (18, 30) that polyglucose particles synthesized by phosphorylase activity alone were amorphous aggregates, stained blue with iodine. On the other hand, newly formed polyglucose particles stained red with iodine were much larger than endogenous glycogen particles. Takeuchi and Miyayama (29) also reported that the size of polyglucose particles synthesized in capillary endothelial and pericytic cells could reach a maximum diameter of over 1400 Å. As pointed out by Iwamasa et al. (10), the macromolecular appearance of these artificial polyglucose particles (26) was very similar in shape to that of endogenous glycogen except in size. It was considered that this size difference in polyglucose particles synthesized from G1-P by the PBG system might result from the conformation of newly formed polyglucoses consisting of α -1,4-glucosidic outer chain and the degree of branching of α -1,6-glucosidic linkage. The small particles probably depend on polyglucose consisting of less branched, longer outer chains and presumably have a tendency to be synthesized by the higher activity of phosphorylase. On the other hand, the larger particles with well branched, shorter outer chains stained red with iodine must be formed by the higher activity of branching glycosyltransferase rather than phosphorylase. However, it is still unclear whether or not polyglucose synthesis under histochemical

FIG. 1. Endogenous glycogen particles (G) in a human gastric cancer cell. They appear as the rosette-like spheroidal branching bodies around lipid droplets in a glycogen area of the cytoplasm. $\times 15,000$

FIG. 2. Endogenous glycogen particles (G) in a human hepatoma cell from HLF can be seen in a certain area of the cytoplasm. $\times 7,500$

FIG. 3. Polyglucose particles (PP) newly synthesized by the PBG system in a human gastric cancer. Particles similar in shape to endogenous glycogen are formed in the expanded glycogen area on the cytoplasm. Their size ranges from 300 Å to 600 Å in diameter. $\times 19,000$

FIG. 4. Higher magnification of synthesized polyglucose particles (PP) in a human gastric cancer. The rosette-like spheroidal branching body can be clearly seen. $\times 50,000$

FIG. 5. Polyglucose particles (PP) histochemically synthesized by the PBG system in a human hepatoma cell. The glycogen area is widely expanded by the polyglucose synthesis in the cytoplasm of a cancer cell. $\times 10,000$

FIG. 6. Newly synthesized polyglucose particles (PP) in a human gastric cancer cell. The same pattern of polyglucose synthesis as in a hepatoma can be seen. $\times 12,500$

FIG. 7. Higher magnification of newly synthesized polyglucose particles (PP) in a human gastric cancer. They show a resemblance in shape to the monoparticulate spheroidal branching body. $\times 50,000$

conditions depends only upon the balance of phosphorylase and branching glycosyl-transferase activities. As pointed out by Ohyumi (15) and Takeuchi et al. (33), a particular synthesis of polyglucose appeared in the cytoplasm of some tissue cells in the shape of a rosary-like string of beads. The rosary-like appearance was very similar in shape to other artificial particles that were histochemically synthesized from uridine diphosphate glucose by UDP-glucose glycogen synthetase activity (32). It is not yet known why a rosary-like string of beads was formed by the phosphorylase reaction in the region of cell matrices under histochemical conditions. It has been pointed out by some investigators (3, 5, 6, 7, 34, 35) that each organ tissue possesses a characteristic phosphorylase isozyme. Actually, newly-formed polyglucoses synthesized from G1-P by the PBG system revealed the characteristic coloration with iodine according to the kind of tissue cells. In the liver, the newly synthesized polyglucose particles appeared as rosette-like spheroidal branching bodies similar to the endogenous glycogen particles, which were usually stained reddish purple with iodine (17, 33). In the heart muscle, monoparticulates or rosary-like strings of beads stained purple with iodine could be synthesized by enzyme activity (15, 29). Monoparticulates of synthesized polyglucoses stained blue with iodine could be recognized in the rat retina (2). These polyglucose particles synthesized by the PBG system seemed to depend on enzyme specificity, which varies according to the tissue cells. Therefore, it is possible that the chemical structure of artificially synthesized polyglucose particles might result from phosphorylase isozyme activity rather than the balance of the PGB system. Further study is needed to clarify the relationship between the different appearances of the newly formed polyglucose particles under the histochemical conditions and its mechanism.

On the other hand, the biological significance of phosphorylase in cancer cells has been histochemically demonstrated by Takeuchi and his co-workers (1, 11, 22, 23, 24, 26, 27). Among the various types, the fetal type of phosphorylase appeared in cancer cells of several organs, particularly in gastric cancers (22, 27). The iodine color of the phosphorylase reaction suggested that the phosphorylase activity was stimulated in some kinds of cancer cells, especially at the phases of DNA-synthesis and mitosis (24). Sato et al. (19) and Iwamasa (9) suggested that the physicochemical property of glycogen in AH-13 cells demonstrated a deviated metabolism of glycogen which resulted from the dysfunction of enzymatic regulation. Actually, glycogen particles of AH-13 cells showed several patterns of particle appearance in size and shape, as previously reported by Iwamasa (9).

Is it histochemically possible to demonstrate the several types of polyglucose particles through deviated phosphorylase activity? Working with the electron-microscope, Sasaki and Takeuchi (16) noticed that in Yoshida ascites hepatoma AH-13 cells, polyglucoses newly formed by enzyme activity appeared as amorphous aggregates and had smaller particles than endogenous glycogen.

In the present study, polyglucose synthesis from G1-P by the PBG-system was carried out in human cancer cells under both the light and electron-microscope. In both the human gastric cancer tissues taken from a surgical gastrectomy, the human gastric cancer cell line cultured in our laboratory and in the human hepatoma cell line from HLF (4), the endogenous glycogen particles appeared in certain areas of the cytoplasm in rosette-like shapes which varied in size (Figs. 1 and 2). They were not very colorfully stained by the iodine reaction. Under the light microscope,

FIG. 8. Polyacrylamide disc-gel electrophoresis patterns of phosphorylase isozymes in some human organ tissues. 1. Skeletal muscle (Sk. Muscle), 2. Liver, 3. Brain, 4. Normal gastric mucosa (Gast. Mucosa), 5. Gastric smooth muscle (Sm. muscle) and 6. Human gastric cancer (G. C.) The skeletal muscle, the liver and the brain respectively show a characteristic patterns of phosphorylase isozymes. A human gastric cancer possesses 2 extra-bands (arrows) except for 2 smooth muscle bands, one of which migrates more rapidly than the smooth muscle and the other one slower.

FIG. 9. Acrylamide disc-gel electrophoresis patterns of phosphorylase isozyme in human gastric cancers. 1. Normal gastric mucosa (Gast. Mucosa), 2. Gastric smooth muscle (Sm. muscle), 3.-6. Human gastric cancers [Case 1. (G. C. 1) Case 2. (G. C. 2) Case 3. (G. C. 3) Case 4 (G. C. 4)] The normal gastric mucosa does not have any band of phosphorylase isozymes. The electrogastric cancer tissues from case 1 to 4 show some variety. Case 1 and 2 show extra-bands (arrows) that are faster and slower than those of the gastric smooth muscle. Case 3 and 4 show only one extra faster band (arrow) other than the smooth muscle one.

the phosphorylase reaction was histochemically demonstrated as varied colors with iodine from reddish to blue in gastric cancer cells as well as the hepatoma. The reaction products were localized diffusely, granularly or massively depending upon the individual cells.

Under the electronmicroscope, the characteristic polyglucose particles were synthesized in the cytoplasm of cancer cells (Figs. 3-7). In human gastric and hepatoma cells, the synthesized polyglucose particles first appeared in the form of rosette-like spheroidal branching bodies (Figs. 3 and 4), similar to endogenous glycogen but larger. Although they were relatively similar in shape, their different size and expanded distribution could suggest to distinguish newly formed poly-

glucoses from endogenous glycogen. The newly formed spheroidal branching bodies were irregular in size, ranging from 300 Å to 600 Å in diameter.

The second pattern of particle appearance was usually identified as that in which the monoparticulates smaller in size were widely distributed in the cytoplasm of cancer cells (Figs. 5, 6 and 7). Their size ranged from 150 Å to 200 Å and was smaller than that of the endogenous muscle glycogen particles (Fig. 7). They were of lower electron-density. This particle difference seems to result in the different iodine coloration in cancer cells. The small monoparticles may be the small spheroidal branching bodies with longer outer chains which are synthesized by the higher phosphorylase activity and stained blue with iodine. The larger spheroidal branching bodies with shorter end groups presumably reveal the higher activity of branching glycosyltransferase.

However, does the different appearance of polyglucose particles synthesized from G1-P by the PBG-system in cancer cells result only from the balance of both phosphorylase and branching glycosyltransferase activities? On the basis of electrophoretic and immunologic experiments employing modified version of the technique of Takeo et al. (21), Sato et al. (33) have recently pointed out that a number of phosphorylase isozymes in ascites hepatoma cells are identical to the fetal type. Working in our laboratory, Hisamitsu also identified the fetal type of phosphorylase isozyme in rat hepatoma cells through the use of his own technique (8).

As reported both by Yonezawa et al. (34) and Sato et al. (20), there are 3 isozymes of phosphorylase in animals: the so-called muscle, liver and fetal (or brain) types.

In the present study, the same results were recognized in the human muscle, liver and brain using Hisamitsu's technique of acrylamide disc-gel electrophoresis, as is shown in Figs. 7 and 8. The human gastric cancers showed a variety of phosphorylase isozymes, while the human normal gastric mucosa did not have any phosphorylase isozyme except for the smooth muscle type. Some of the gastric cancer tissues contained electrophoretically similar bands to those of the smooth muscle and brain (or fetal) isozymes, although this similarity is difficult to identify with the sole use of the electrophoretic migration method. A human hepatoma cell contained 2 bands different from the normal adult liver and one which was similar to the fetal liver isozyme. These isozymes should be studied in the future through further immunological investigation.

From the above mentioned findings, it would seem that the iodine coloration and appearance of synthesized spheroidal branching bodies results not only from the activity balance of the enzyme system, but also from the differences in isozymes found in cancer cells. These results may depend on the dysfunction of normal regulation in the cell metabolism and deviation of enzymes in cancer cells. Many unknown factors still remain to be clarified in the future.

REFERENCES

1. Akagi, M. Shiraishi, Y. and Takeuchi, T.: Histochemical properties of mucous changes in stomach cancer and its borderline cases. *Japan J. Cancer Clin.* 18; 850-858, 1972.
2. Amemiya, T.: Intracellular polysaccharide synthesis in Müller cells of the normal rat retina, an electron microscopic study. *J. Histochem. Cytochem.* 3; 41, 1970.
3. Davis, C. H., Schlisfeld, L. H., Wolf, D. P., Leavitt, C. A. and Krebs, E. G.: Interrelation-

- ships among glycogen phosphorylase isozymes. *J. Biol. Chem.* 242; 4824, 1967.
4. Doi, I., Namba, M. and Sato, J.: Establishment and some biological characteristics of human hepatoma cell lines. *Gann.* 66; 385-392, 1975.
 5. Hanabusa, K. and Kohno, H.: Isolation and comparison of phosphorylase b isozymes in pig heart. *J. Biochem. (Tokyo)* 66; 69, 1969.
 6. Hanabusa, K., Kohno, H. and Matsuyama, S.: Electrofocusing properties of pig phosphorylase isozymes. *J. Biochem. (Tokyo)* 72; 1261, 1972.
 7. Henion, H. F. and Sutherland, E. W.: Immunological difference of phosphorylases. *J. Biol. Chem.* 224; 477, 1957.
 8. Hisamitsu, S. and Takeuchi, T.: Improved disc gel electrophoretic method of glycogen phosphorylase isozymes. *Acta histochem. Cytochem.* 9; 102, 1976.
 9. Iwamasa, T.: Ultrastructure and physico-chemical properties of glycogen from ascites hepatoma AH 13. *Acta histochem. cytochem.* 5; 117-124, 1972.
 10. Iwamasa, T., Fujisaki, A. and Takeuchi, T.: Ultrastructure of glycogen particles, a study from the ultrastructural and physico-chemical view points. *J. Electron Microsc.* 19; 371, 1971.
 11. Kinoshita, K.: Histochemical demonstration of amylophosphorylase in tumor tissue cells. *Igaku Kenkyu* 27; 1689-1732, 1957.
 12. Miyayama, H.: The light and electron microscopic observation of polyglucose histochemically synthesized in the rat skeletal muscles by enzymes, particularly phosphorylase and branching glycosyltransferase. *Acta histochem. cytochem.* 5; 1, 1972.
 13. Miyayama, H. and Takeuchi, T.: Polyglucose particles synthesized by phosphorylase and branching glycosyltransferase in rat skeletal muscle fibers. *J. Electron Microsc.* 22; 63, 1973.
 14. Miyayama, H., Okabe, M., Shigenaga, K. and Takeuchi, T.: Intracellular synthesis of polyglucose particles under histochemical conditions in the normal rat. *Acta histochem. cytochem.* 8; 247-256, 1975.
 15. Ohyumi, M.: Histochemical and ultrastructural studies of enzymes, particularly in carbohydrate metabolism of the bovine heart conduction system. *Acta histochem. cytochem.* 8; 164, 1975.
 16. Sasaki, M. and Takeuchi, T.: Electron-histochemical studies on the lysosome system ascites hepatoma AH 13 cells. *Acta Path. Jap.* 19; 443, 1969.
 17. Sasaki, M., and Takeuchi, T.: Electron histochemical observation of polysaccharide synthesized by enzyme activities in rat liver cells. *Acta histochem. cytochem.* 1; 197, 1968.
 18. Sasaki, M. and Takeuchi, T.: Histochemical and electron microscopic observation of glycogen synthesized from glucose-1-phosphate by phosphorylase and branching enzyme in human muscle. *J. Histochem. Cytochem.* 11; 342-348, 1963.
 19. Sato, K., Abe, N. and Tsuiki, S.: Comparison of glycogen synthetase of ascites hepatomas with that of liver and the effect of glycogen on its macromolecular conversion. The 29th annual meeting of the Japanese Cancer Association. 1971, p. 10.
 20. Sato, K., Satoh, K., Sato, T., Imai, F. and Morris, H. P.: Isozyme pattern of glycogen phosphorylase in rat tissues and transplantable hepatomas. *Cancer Res.* 36; 487-495, 1976.
 21. Takeo, K. and Nakamura, S.: Dissociation constants of glucan phosphorylases of rabbit tissues studied by polyacrylamide gel disc electrophoresis. *Arch. Biochem. Biophys.* 153; 1-7, 1972.
 22. Takeuchi, T.: Biological significance of phosphorylase from view point of proliferation and differentiation of tissue cells. *Symp. Cell Chem.* 14; 137, 1964.
 23. Takeuchi, T.: Diagnosis of cancer cell from histochemical point of view. *Symp. The 17th annual meeting of Japanese medical association.* 1; 448-460, 1967.
 24. Takeuchi, T.: Enzyme-histochemical and autoradiographic observations on cyclic glycogen metabolism. Summary Report of Third Intern. Cong. of Histochem. Cytochem. New York. 1968, pp. 266-267.
 25. Takeuchi, T.: Morphological changes of cancer cells in vivo and in vitro. Histochemical and electron microscopical observations. *Nippon Rinsho* 29; 95, 1971.

26. Takeuchi, T.: Ultrafine structure of glycogen particles synthesized under histochemical conditions. *J. Histochem. Cytochem.* 22; 378, 1974.
27. Takeuchi, T., Kinoshita, K. and Kobayakawa, S.: Characteristics of phosphorylase reaction in tumor cells. *Tokyo Iji Shinshi* 73; 282-284, 1956.
28. Takeuchi, T. and Kuriaki, H.: Histochemical detection of phosphorylase in animal tissues. *J. Histochem. Cytochem.* 6; 208-216, 1958.
29. Takeuchi, T. and Miyayama, H.: Macromolecular particles of polyglucose synthesized under histochemical conditions in the endothelial cells of rabbit blood vessels. *Histochem. J.* 5; 451, 1973.
30. Takeuchi, T. and Sasaki, M.: Electron microscopical observation of polyglucose synthesized from glucose-1-phosphate by the phosphorylase activity in rat skeletal muscle. *Histochemie* 23; 310, 1970.
31. Takeuchi, T. and Sasaki, M.: Histochemical and electron microscopic differences between native glycogen and polyglucose synthesized by phosphorylase in tissue cells. *Acta histochem. cytochem.* 1; 63, 1968.
32. Takeuchi, T., Sasaki, M. and Matsumoto, O.: An electron histochemical observation of polysaccharide synthesized from uridine diphosphoglucose by the enzyme activity. *Acta histochem. cytochem.* 3; 173, 1970.
33. Takeuchi, T., Sasaki, M., Miyayama, H., Ohyumi, M. and Miyajima, H.: Intracellular localization and size of glycogen particles in glycogen synthesized under histochemical condition. *J. Histochem. Cytochem.* 12; 945, 1975.
34. Yonezawa, S. and Hori, S.H.: Electrophoretic studies on the phosphorylase isozymes. *J. Histochem. Cytochem.* 23; 745-751, 1975.
35. Yunis, A. A., Fisher, E. H. and Krebs, E. G.: Comparative studies on glycogen phosphorylase. IV. Purification and properties of rabbit heart phosphorylase. *J. Biol. Chem.* 237; 2809, 1962.