Short Communications

SC 11082

UDPG–glycogen transglucosylase of the human leukocyte

Evidence has been presented by LELOIR and coworkers[1,2] that glycogen is synthesized from uridine diphosphate glucose (UDPG) in the liver and skeletal muscle. These findings thus led to the belief that the synthesis of glycogen occurs primarily through UDPG–glycogen transglucosylase (UDP glucose: α-1,4-glucan α-4-glucosyltransferase, EC 2.4.1.11) and independently of the phosphorylase (α-glucan phosphorylase, E.C. 2.4.1.1) reaction which is now considered to be primarily concerned with the catabolism of the polysaccharide. Support for this concept is found in the work of several investigators[3–5] who have demonstrated that a lack of phosphorylase accounts for the defect in a form of glycogen-storage disease characterized by myopathy. HÜLSMANN *et al.*[6] have demonstrated a deficiency of phosphorylase in leukocytes of patients with liver phosphorylase deficiency. WILLIAMS AND FIELD[7] have also examined the leukocytes of patients with glycogen-storage disease of the hepatic-phosphorylase-deficient type and found that these cells also reflect a deficiency of phosphorylase activity in spite of normal glycogen concentrations. This, they felt, was proof of independent pathways for the synthesis and degradation of glycogen. TAKEUCHI, TADOKORO AND IDE[8] however, were unable to demonstrate the presence of UDPG–glycogen transglucosylase in the leukocyte.

Since this laboratory has been interested in the glycogen metabolism of the leukocyte, it was of interest to determine whether this pathway does exist in these cells.

Blood samples were obtained from patients admitted to the hospital for minor surgical procedures. Their cell and differential counts were normal. Leukocytes were separated from citrated whole blood by differential sedimentation in a saline solution of polyvinylpyrrolidone (5%) and bovine fibrinogen (2%) at a ratio of 2 parts blood to one part of the polyvinylpyrrolidone–fibrinogen mixture. The red cells were allowed to settle and the leukocyte-rich supernatant was decanted and centrifuged for 10 min at approx. 1000 $\times$ g at 4°. The cells were washed with normal saline three times prior to use. After the final wash the cells were resuspended in saline to approximately one half the original volume of whole blood. Leukocyte counts were made to determine the number of cells in suspension. A known number of cells was centrifuged out and resuspended in a sucrose (0.25 M)–EDTA (0.001 M) mixture. The cells were then lysed by repeated freezing and thawing.

Aliquots of the lysed cell suspension were assayed for the transglucosylase activity by the method of LELOIR AND GOLDEMBERG[9].

With the liver enzyme, LELOIR found that the relationship between UDP formation and time or enzyme concentration was linear when the UDP formed did not exceed 0.06 μmole.

Preliminary experiments in this laboratory indicated that for the range of

leukocyte concentration used, the formation of UDP was linear up to 30 min. In these experiments the UDP formation in 15 min was used for the assay of activity.

The activity of normal leukocytes expressed as UDP released, averaged 0.21 ± 0.06 μmole UDP per 10^7 white blood cells in 15 min. The values of 15 individual samples ranged from 0.12 to 0.32 μmole per 10^7 white blood cells in 15 min.

It was found that the activity of the enzyme must be measured as rapidly as possible after the blood has been withdrawn from the patient and the cells separated. When whole blood was allowed to stand at room temperatures for a period of 2 h before separating the cells, the activity of the enzyme increased an average of 42% over the level found when the cells were separated and assayed immediately after withdrawal of the sample from the patient. The enzyme also proved to be somewhat labile when the blood was stored for 2 h at 4° prior to separating the cells. Under these conditions, the activity was found to be decreased approx. 20–25% in most instances. In a few samples the activity remained unchanged. The reason for these changes is not clear at present. Correlation between the activity of the enzymes with the total glycogen content of these cells was difficult. There appeared to be no consistent pattern in this respect.

The results of these experiments have demonstrated that UDPG–glycogen transglucosylase is also present in the leukocyte and agree with the observations made on other tissues that independent pathways exist for glycogen synthesis and degradation. The inability of TAKEUCHI *et al.*[8] to demonstrate this pathway in the leukocyte histochemically may lie in the conditions under which their assays were performed.

In two cases of chronic myeloid leukemia, with leukocyte glycogen contents of 34 and 29 μg per 10^7 white blood cells, the enzyme activities were 0.19 and 0.22 μmole UDP per 10^7 white blood cells in 15 min, both of which are within the normal range. These findings are in agreement with some of our other studies from which we have concluded that the low level of glycogen found in the chronic myeloid leukemic leukocyte is probably not a deficiency of any of the enzymes required for the synthesis of glycogen in these cells.

This work was supported in part by U.S. Public Health Service grant C-6463.

Medical Research Laboratories,
Veterans Administration Hospital,
and Department of Biology,
Upsala College,
East Orange, N.J. (U.S.A.)

WARREN L. MILLER
CHRISTINA VANDERWENDE

[1] L. F. LELOIR AND C. E. CARDINI, *J. Am. Chem. Soc.*, 79 (1957) 6340.
[2] L. F. LELOIR, J. M. OLIVARRIA, S. H. GOLDEMBERG AND H. CARMINATTI, *Arch. Biochem. Biophys.*, 81 (1959) 508.
[3] R. SCHMID, P. W. ROBBINS AND R. R. TROUT, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1236.
[4] W. F. H. M. MOMMAERTS, B. ILLINGWORTH, C. M. PEARSON, R. J. GUILLORY AND K. SERAYDARIAN, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 791.
[5] J. LARNER AND C. VILLAR-PALASI, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1234.
[6] W. C. HÜLSMANN, T. L. OEI AND S. VAN CREVELD, 2 *Lancet*, (1961) 581.
[7] H. E. WILLIAMS AND J. B. FIELD, *J. Clin. Invest.*, 40 (1961) 1841.
[8] T. TAKEUCHI, N. TADOKORO AND H. IDE, *J. Histochem. Cytochem.*, 10 (1962) 572.
[9] L. F. LELOIR AND S. H. GOLDEMBERG, *J. Biol. Chem.*, 235 (1960) 919.

Received April 17th, 1963