AMYLO-1,6-GLUCOSIDASE IN HUMAN FIBROBLASTS: STUDIES IN TYPE III GLYCOGEN STORAGE DISEASE

Parvin Justice, Catherine Ryan, David Yi-Yung Hsia

Departments of Pediatrics and Biochemistry, Loyola University-Stritch School of Medicine, Maywood, Illinois 60153

and Eva Krmpotik

Department of Pathology, Chicago Medical School University of Health and Sciences, Chicago, Illinois

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SUMMARY

Using the assay system which measures the incorporation of U-C^{14} glucose into new branch points in the glycogen molecule, it has been possible to show the presence of amylo-1,6-glucosidase in human fibroblasts derived from skin biopsies and in cultivated amniotic fluid cells. The marked decrease of this enzyme in cultured fibroblasts from a child with Type III glycogen storage disease indicate the usefulness of this technique not only in establishing the diagnosis among patients, but opens up the possibility of prenatal detection of debrancher enzyme deficiency.

The studies of Abdullah and Whelan (1) and Brown, Illingworth, and Cori (2) have shown that the debranching enzyme system catalyzing the degradation of branching points in glycogen involves two steps: first, the uncovering of the branching point by maltotriosyl-transferase, and second, the hydrolysis of the 1,6-glucosidic linkage, producing glucose, by amylo-1,6-glucosidase. The latter enzyme may be assayed either by the "reverse" reaction measuring the incorporation of U-C^{14} glucose into new branch points in the glycogen molecule (3), or by the "forward" reaction estimating the release of glucose from phosphorylase limit dextrin (4).

During the past decade, a number of reports (5-9) have appeared showing a marked deficiency or absence of amylo-1,6-glucosidase using either or both assay methods in the liver, muscle, erythrocytes, and leukocytes among patients who are homozygous for

Type III glycogen storage disease. Furthermore, Chayoth and his coworkers (10) have demonstrated a 50 per cent decrease of erythrocyte and leukocyte amylo-1,6-glucosidase activity among heterozygous carriers for Type III glycogen storage disease using the U-C^{14} method, but Huijing and his coworkers (11) were unable to demonstrate any decrease among similar carriers using the limit dextrin method.

In this paper, we shall report the presence of amylo-1,6-glucosidase in both fibroblasts derived from skin biopsies and in cultivated amniotic fluid cells and describe some observations made on a family with Type III glycogen storage disease.

METHODS AND MATERIALS

For these studies, amylo-1,6-glucosidase activity was assayed according to the method of Hers (3). The incubation mixture consisted of 0.2 ml of 20 per cent glycogen, 0.075 ml D-glucose U-C^{14}, 20 uc/ml .244 mg glucose (New England Nuclear), 0.05 ml 0.5 M sodium fluoride, 0.1 M phosphate buffer, pH 7.4, and 0.1 ml cell lysate. The incubation was for two hours at 37 C and the reaction was stopped by adding 6.5 ml of a solution containing 0.5 ml 20 per cent TCA, 4 ml ethanol, and 2 ml water. The precipitate was collected by centrifugation and then redissolved in 2 ml distilled water. After centrifugation the precipitate was dissolved in 2 ml 20 per cent potassium hydroxide and was heated in a boiling water bath for 30 minutes. The glycogen was then reprecipitated three times with 2.4 ml alcohol and redissolved in 2 ml H_2O. The final precipitate was added to .5 ml water and the entire sample was counted in a liquid scintillation counter. An appropriate blank in which the reaction was stopped immediately after addition of cell lysate was carried out through the entire procedure.

Studies were first carried out in normal fibroblasts derived from skin biopsies and cultured in monolayers using a growth media containing 20 per cent fetal calf serum and antibiotics. With fibroblasts, the incorporation of radioactive substrate into limit dextrin was a linear function of time of incubation and of the enzyme concentration and the amylo-1,6-glucosidase activity from 8 control cultures showed a range of 230 to 820 cpm/mg protein/hr.

Studies were next carried out in cultivated amniotic fluid cells obtained by abdominal amniocentesis from a woman in the 16th week of gestation. Amylo-1,6-glucosidase from 3 cultures showed a range of 1200 - 3500 cpm/mg protein/hr, which is higher than that of normal fibroblasts.

We have had the opportunity to study a patient with Type III glycogen storage disease. This negro male was born on September 19, 1963 and first admitted to the hospital because of an enlarged liver at the age of 16 months. This child had mild jaundice with a direct bilirubin of 2.7 mg% and an indirect bilirubin of 0.32 mg%. The SGOT was 75 and the SGPT was 27. Serum cholesterol was 495 mg%. The lipid studies performed on this patient have been previously reported by Jakovcic, Khachadurian and Hsia (12). The diagnosis of Type III glycogen storage disease was established by biochemical studies* which showed 13.6 per cent glycogen in liver and 5.6 per cent glycogen in muscle. The outer chain was degraded 17 per cent in liver and 6 per cent in muscle by purified phosphorylase. Amylo-1,6-glucosidase was negative in both liver and muscle. Liver and muscle phosphorylase, glucose-6-phosphatase, and phosphofructokinase were normal.

* Kindly performed by Dr. Barbara Brown, Department of Biochemistry, Washington University, St. Louis, Missouri.

TABLE I Leukocyte amylo-1,6-glucosidase activity

SUBJECTS	CPM/hr/10^8WBC
Control	4042
Mother (GSD III)	1650
Patient (GSD III)	33

TABLE II Fibroblast amylo-1,6-glucosidase activity

SUBJECTS	CPM/hr/mg/protein
Cultured normal fibroblasts	280 - 820
Cultivated amniotic fluid cells	1200 - 3500
Patient (GSD III)	69
Mother (GSD III)	610
Patient (GSD I)	515

RESULTS

Blood was obtained from this patient, his mother, and a normal control and the leukocytes were separated from the erythrocytes by fibrinogen sedimentation. As shown in Table I the leukocyte amylo-1,6-glucosidase was virtually absent in the patient and about half of normal in his mother confirming the previous observations of Chayoth and his coworkers (10) using the U-C^{14} method.

Skin biopsies were obtained from the patient and his mother and enzyme activity was determined in the fibroblasts grown in

culture. As shown in Table II, the fibroblast amylo-1,6-glucosidase activity in the patient was much lower than the lower limits of the normal range, but the mother and a patient with proven Type I glycogen storage disease showed debrancher enzyme activity within the normal range. Glucose-6-phosphate dehydrogenase activity in fibroblasts was used as a marker enzyme and this was found to be within the normal range both in the patient and his mother.

DISCUSSION

The present assay system is based on the principle that amylo-1,6-glucosidase splits only 1,6 linked glucose units from glycogen *in vivo*. *In vitro*, the enzyme reaction is reversible, and it is on this basis that the assay system was established, namely, the incorporation of glucose-C^{14} into glycogen only at these branch points, or the reverse of what happens *in vivo*. Hers (3) has demonstrated in liver that the radioactive glucose is incorporated into glycogen only at branch points. If the incorporation of radioactivity into glycogen were proceeding via the uridine diphosphate glucose glycogen synthetase pathway or as a result of phosphorylase activity, the radioactivity in the glycogen, should not be restricted to the glucose molecules at the branch points. Although this was not proven in fibroblasts in the present study, presumably the enzyme in this tissue is similar to that in human liver.

The present study does not indicate whether the introduction of radioactive glucose at the branch point results from an exchange reaction or whether there is actual net synthesis of glycogen during the reaction.

Finally, the presence of debranching in cultivated amniotic fluid cells opens up the possibility of prenatal detection of

Type III glycogen storage disease.

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