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A Rapid Electrophoretic Technique for Human and Chinese Hamster Galactokinase

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Summary. In this paper a simple direct staining method is described for the detection of the human and Chinese hamster forms of galactokinase as separated by electrophoresis.

Galactokinase (EC 2.7.1.6) plays a key role in the utilization of galactose by catalyzing the conversion of galactose to galactose-1-phosphate (gal-1-P) in the presence of Mg^{2+} and adenosine triphosphate (ATP). The great bulk of galactose metabolized appears to follow the Leloir pathway (Beutler and Morrison, 1967). Galactokinase deficiency galactosemia in man is inherited as an autosomal recessive trait and results in gastrointestinal dysfuction and jaundice in the newborn period and the appearance of cataracts in the older patients on unrestricted diets (Gitzelmann, 1967; Segal, 1972). The gene for galactokinase has been recently assigned to a specific region (q21—22) of the human chromosome 17 (Elsevier *et al.*, in press). The assignment of the gene was made possible by the development of an electrophoretic method for separating the homologous enzyme derived from human, Chinese hamster and mouse tissues (Nichols *et al.*, 1974). The technique is based on the precipitation of ^{14}C-D-galactose-1-PO_4 into the matrix of the gel by lanthanum chloride. The gel is then washed to remove unreacted ^{14}C-D-galactose, dried, exposed to X-ray film where the site of enzyme activity is demonstrated by autoradiography. The method appears to be sensitive but is laborious and time consuming. In this paper we describe a simple, direct staining method for the detection of galactokinase on starch gel. The introduction of this simplified method may facilitate gene mapping and linkage studies by the somatic cell genetic techniques. When extended to erythrocytes, the method may also be applied to the search for polymorphism of this enzyme in human populations.

Materials and Methods

About 6×10^6 tissue culture cells were collected by trypsinization, washed three times with isotonic saline, lysed in 1 ml double-distilled water and sonicated for 30 sec in an ice-bath.

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The sonicated samples were then centrifuged at 20000 g for 20 min at 4° C to remove cell debris. After centrifugation the lipid layer was pipetted off and the clear supernatant retained either for immediate assay or stored in liquid nitrogen.

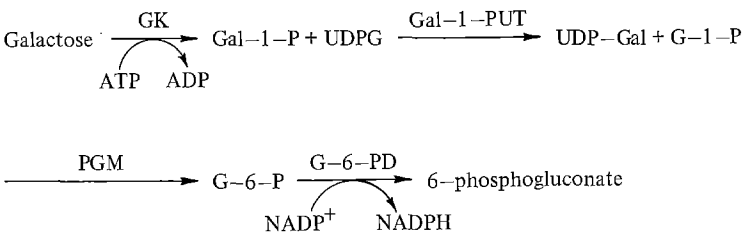
The cell extract was run by vertical electrophoresis at pH 7.0 in L-histidine gel (gel buffer: 5 mM L-histidine-HCl; electrode buffer: 0.41 M sodium citrate adjusted with 0.41 M citric acid to pH 7.0) made of 55 g/400 ml Electrostarch (Electrostarch Co., Madison, Wisconsin). The electrophoresis was performed at 8 V/cm at 4° C for 5 hrs. After the run, a 7 cm section of the gel anodal to the origin was cut from each gel and sliced in half. Both halves were eased into a 15 × 10.5 × 1 cm plastic tray and incubated at 37° C with 20 ml of a staining mixture prepared as follows. All biochemicals and enzymes were obtained from Sigma Co.

Tris-HCl (1 M, pH 7.6), $MgCl_2 \cdot 6H_2O$ (50 mM), Glucose 1,6-diphosphate (60 μM), NADP (10 mM), UDPG (5 mM), ATP (60 mM), PMS (0.1 mg/ml), MTT tetrazolium (4.0 mg/ml), Gal-1-PUT (5 units/ml), PGM (5 units/ml), G-6-PD (5 units/ml), Galactose (4 mM).

After incubation of the gel at 37° C in the dark for 1 hr, bands of galactokinase appear as dark blue zones against a white background.

Results and Discussion

In this staining method galactokinase (GK) catalyzes the conversion of galactose to galactose-l-phosphate which in turn is converted by Gal-1-P uridyltransferase (Gal-1-PUT, EC 2.7.7.12) to glucose-l-phosphate. The oxidation of glucose-1-phosphate to 6-phosphogluconate via glucose-6-phosphate takes place by coupling the reaction with phosphoglucomutase (PGM, EC 2.7.5.1) and glucose-6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49). The oxidation is coupled to the reduction of MTT tetrazolium to blue formazan in the presence of phenazine methosulfate (PMS). The reactions involved in the detection of galactokinase is shown in the following.



The zymographic patterns for galactokinase of human, Chinese hamster, and a number of hybrid cell lines formed after fusion between cells of the 2 species are presented in Fig. 1. The origin and properties of these cells and hybrids have been described elsewhere (Sun *et al.*, 1974). It can be seen from Fig. 1 that the human form of galactokinase can be distinguished from that of the Chinese hamster by its slower anodal movement during electrophoresis, a reflection of possible difference in the amino acid composition of the homologous enzymes from the two species. The validity of the staining method was tested by omitting each of the reagents from the reaction mixture. All were found to be essential for the bands of activity to appear.

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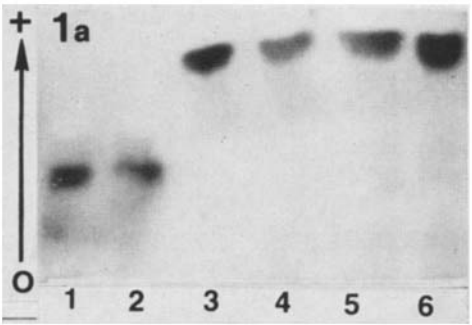

Fig. 1. Zymogram showing the activity of galactokinase from the following cell lines: channel 1, UM-10, a human lymphoblastoid cell line from a male donor with Lesch-Nyhan syndrome; channel 2, P_3J, a human Burkitt lymphoma cell line; channel 3, GJ-1, a human-Chinese hamster cell hybrid containing one complete set of Chinese hamster chromosomes characteristic of V79 cell line and a single human chromosome A2. This hybrid was formed between P_3J cell and a hamster mutant cell gal-2 which cannot utilize exogenous galactose; channel 4, GM-1, a hybrid formed after fusion of gal-2 and UM-10 cells; channel 5, gal-2; channel 6, GJ-3, a hybrid between P_3J and another similar hamster mutant cell line gal-3. Human chromosome 17 was absent in all 4 interspecific cell hybrid lines

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