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A STUDY OF GALACTOKINASE AND GLUCOSE 4-EPIMERASE FROM NORMAL AND GALACTOSEMIC SKIN FIBROBLASTS

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SUMMARY

Galactokinase and epimerase from skin fibroblasts of control and galactosemic individuals were studied with respect to specific activity, pH optimum, K_m values and thermal stability. The properties of galactokinase and epimerase were similar in control and galactosemic fibroblasts. These data indicate that the mutation at the transferase locus does not alter either the level or the nature of galactokinase and epimerase from skin fibroblasts of control and galactosemic individuals.

INTRODUCTION

Galactosemia, an autosomal recessive inherited defect, is caused by the deficiency of the enzyme galactose 1-phosphate uridyltransferase (UDPglucose: α-D-galactose-1-phosphate uridyltransferase, EC 2.7.7.12, transferase)[1]. Deficiency of transferase results in impaired utilization of ingested galactose, leading to the accumulation of galactose 1-phosphate in tissues[2]. In spite of the enzymatic defect, metabolism of small amounts of galactose can be demonstrated in patients with the disorder[3]. A number of possible alternate pathways of galactose metabolism have been reported[4-9]. We have previously reported that human skin fibroblasts derived from control and galactosemic individuals contain an alternate pathway to metabolize galactose 1-phosphate *via* the UDPGal pyrophosphorylase reaction[10]. In addition, we have also demonstrated that the mutation on the transferase locus did not have any effect on the biochemical properties of UDPGal pyrophosphorylase and UDPGlc pyrophosphorylase. The purpose of this study was to investigate the effect of mutation at the transferase locus on the biochemical properties of galactokinase (ATP:D-galactose 1-phosphotransferase, EC 2.7.1.6) and UDPglucose epimerase (UDPglucose 4-epimerase, EC 5.1.3.2, epimerase) of skin fibroblasts obtained from normal and galactosemic individuals under various conditions of culture. These enzymes were studied with respect to specific activity, Michaelis–Menten constants, thermal stability and pH optima.

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MATERIALS AND METHODS

Skin biopsies were obtained from eight patients with galactosemia and age matched controls by techniques previously described[11]. Fibroblasts were cultivated and processed as described earlier[12]. The cell suspensions in appropriate buffers (8 mM Tris–HCl, pH 7.8, for galactokinase and 10 mM glycine–NaOH buffer (pH 8.7) containing 0.1 mM NAD^+ for epimerase) were disrupted by repeated freezing and thawing. The lysates were centrifuged at 60 000 $\times$ g for 30 min at 4 °C and the supernatants were used for assays.

Galactokinase activity was measured by the method of Sherman[13]. The epimerase assay was performed by modifying the two step reaction[14]. The UDPGlc formed by the action of epimerase on UDPGal is quantitatively oxidized by UDP-Glc dehydrogenase and NAD^+. The rate of NADH formation is a measure of epimerase activity. Protein was determined by the method of Lowry *et al.*[15].

RESULTS AND DISCUSSION

Specific activities of galactokinase and epimerase were similar in both control and galactosemic cell lines (Table I). During repeated subculture and various phases of growth the specific activities remained unchanged. Both control and galactosemic fibroblasts could be grown in media containing 75 mg/100 ml galactose and 25 mg/100 ml glucose, 50 mg/100 ml each galactose and glucose, and 25 mg/100 ml galactose and 75 mg/100 ml glucose. In contrast, when fibroblasts were grown in media containing 100 mg/100 ml galactose in place of 100 mg/100 ml glucose the galactosemic

TABLE I

SPECIFIC ACTIVITIES OF GALACTOKINASE AND EPIMERASE OF SKIN FIBROBLASTS FROM NORMAL AND GALACTOSEMIC PATIENTS

The assay mixture for galactokinase contained 200 nmoles [1-^{14}C]galactose ($7.0 \cdot 10^4$–$8.0 \cdot 10^4$ cpm) 300 nmoles ATP, 800 nmoles $MgCl_2$, 640 nmoles NaF, 2.0 μmoles dithiothreitol, 20 μmoles Tris–HCl buffer (pH 7.8) and fibroblast lysate containing 15–100 μg protein in a total volume of 0.20 ml. The reactions were carried out at 37 °C for 30 min. 50-μl samples were taken at the beginning and end of incubation, spotted on circular (3.5 cm diameter) DE 81 paper and washed immediately with 1 l of deionized water. The papers were dried with a stream of hot air and counted at 85% efficiency in 15 ml PPO–POPOP (4.0 g PPO and 0.1 g POPOP per 1 of toluene) using a Mark II Nuclear Chicago, liquid scintillation spectrometer. Specific activity is expressed as μmoles galactose phosphorylated/h per mg protein. The values in parentheses indicate the number of determinations. The reaction mixture for epimerase in a total volume of 1.0 ml contained 0.5 μmole UDPGal, 0.1 μmole cysteine, 1.0 μmole NAD^+ (pH 7.4), 100 units of UDPGlc dehydrogenase, 200 μmoles glycine–NaOH buffer (pH 8.7) and fibroblast lysate equivalent to 20–300 μg protein. The reaction was started by the addition of enzyme and the rate of NADH production was recorded for 10–15 min at 37 °C using a Gilford Model 2000 spectrophotometer. The specific activity was then calculated taking into consideration that for each mole of UDPGlc oxidized 2 moles of NADH were produced and expressed as μmoles of UDPGlc formed/h per mg protein.

Enzyme	*Cell line*	
	Normal	*Galactosemic*
Galactokinase	0.104 ± 0.035 (19)	0.114 ± 0.040 (18)
Epimerase	0.402 ± 0.141 (30)	0.325 ± 0.186 (17)

TABLE II

MICHAELIS–MENTEN CONSTANTS FOR GALACTOKINASE AND EPIMERASE FROM SKIN FIBROBLASTS OF NORMAL AND GALACTOSEMIC INDIVIDUALS

The assays were carried out as indicated in Table I. K_m values for galactokinase and epimerase were calculated from Lineweaver–Burk plots. The concentrations of substrates used were as shown in the text. The values in the parentheses indicate number of determinations.

	Cell line	
	Normal	*Galactosemic*
Galactokinase		
K_m galactose at 1.5 mM ATP	$1.95 \cdot 10^{-3} \pm 0.73 \cdot 10^{-3}$ M (4)	$1.56 \cdot 10^{-3} \pm 0.31 \cdot 10^{-3}$ M (4)
K_m ATP at 1.0 mM galactose	$3.30 \cdot 10^{-5} \pm 1.15 \cdot 10^{-5}$ M (5)	$2.64 \cdot 10^{-5} \pm 1.16 \cdot 10^{-5}$ M (5)
Epimerase		
K_m UDPGal	$4.92 \cdot 10^{-5} \pm 1.74 \cdot 10^{-5}$ M (8)	$4.20 \cdot 10^{-5} \pm 0.79 \cdot 10^{-5}$ M (4)

cells failed to grow after 72 h. Substituting the glucose completely or partially by galactose did not have any effect on the specific activities of galactokinase and epimerase in control or galactosemic fibroblasts.

The K_m values for galactokinase at pH 7.8 for galactose (0.2–1.0 mM) at 1.5 mM ATP concentration and for ATP (0.03–0.3 mM) at 1.0 mM galactose concentrations were similar in control and galactosemic fibroblasts (Table II). Similarly the K_m for epimerase at pH 8.7 for UDPGal (0.02–0.6 mM) did not differ in control and galactosemic cell lines.

Galactokinase activity determinations in 100 mM Tris–HCl buffers of pH range 7.0–8.8 exhibited maximal activity at pH 7.8 for both control and galactosemic fibroblasts. The epimerase in control and galactosemic fibroblasts had an optimum

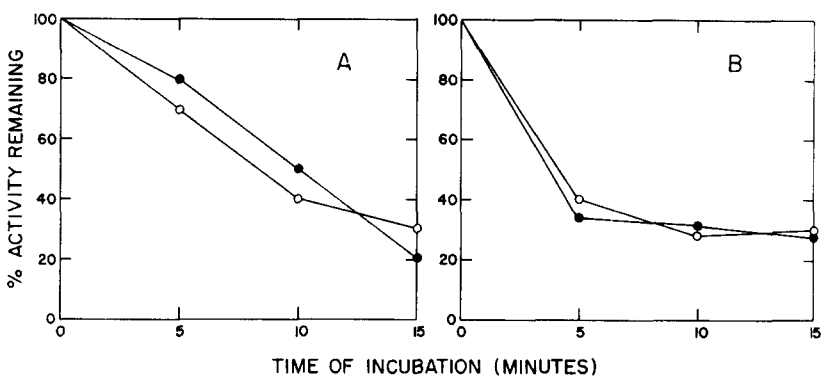

Fig. 1. Effect of incubation at 47 °C on galactokinase and epimerase from fibroblasts of control and galactosemic individuals. The high speed supernatants were adjusted to 2 mg protein/ml and incubated at 47 °C for 0, 5, 10 and 15 min. The samples were chilled immediately and galactokinase and epimerase assays were carried out on the samples as described in Table I. Activity remaining at the end of incubation was calculated as percent activity of 0 time and plotted against time of incubation. The plots are the average results of four separate experiments. (A) Galactokinase activity. ●—●, galactosemic fibroblasts; ○—○, control fibroblasts. (B) Epimerase activity. ●—●, galactosemic fibroblasts; ○—○, control fibroblasts.

between pH values of 8.7–9.0 (200 mM glycine–NaOH buffers, pH range 7.8–10.0).

When galactokinase and epimerase activities were measured after incubating the lysates (2 mg protein/ml) at 47 °C for 0, 5, 10 and 15 min, the loss of activity for both galactokinase and epimerase was of the same magnitude in control and galactosemic fibroblasts (Figs 1A and 1B).

These studies clearly indicate that the specific activity, pH optimum, thermal stability and K_m values are similar for galactokinase in control and galactosemic fibroblasts. These parameters for epimerase are also similar in control and galactosemic cell lines. Stifel *et al.*[16] have demonstrated an 8-fold increase in galactokinase and transferase and 4-fold increase in epimerase activities in the jejunum of galactose fed rats as compared to fasting rats. In contrast our studies on fibroblasts grown in different concentrations of galactose under our culture conditions did not show any increase in activities of these enzymes in control or galactosemic fibroblasts. Apparently the level of these enzymes is not regulated by galactose in both control and mutant fibroblasts. Thus the mutation at the transferase locus appears to have no significant effect either on the nature or level of these two enzymes.

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Henry L. Nadler is the Given Research Professor of Pediatrics.

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